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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 81, NO. 2, PP. 95–97 MARCH, 1954

CROSS-KARYOGAMY AND SEGREGATION IN A HOMOTHALLIC FUNGUS

LINDSAY S. OLIVE

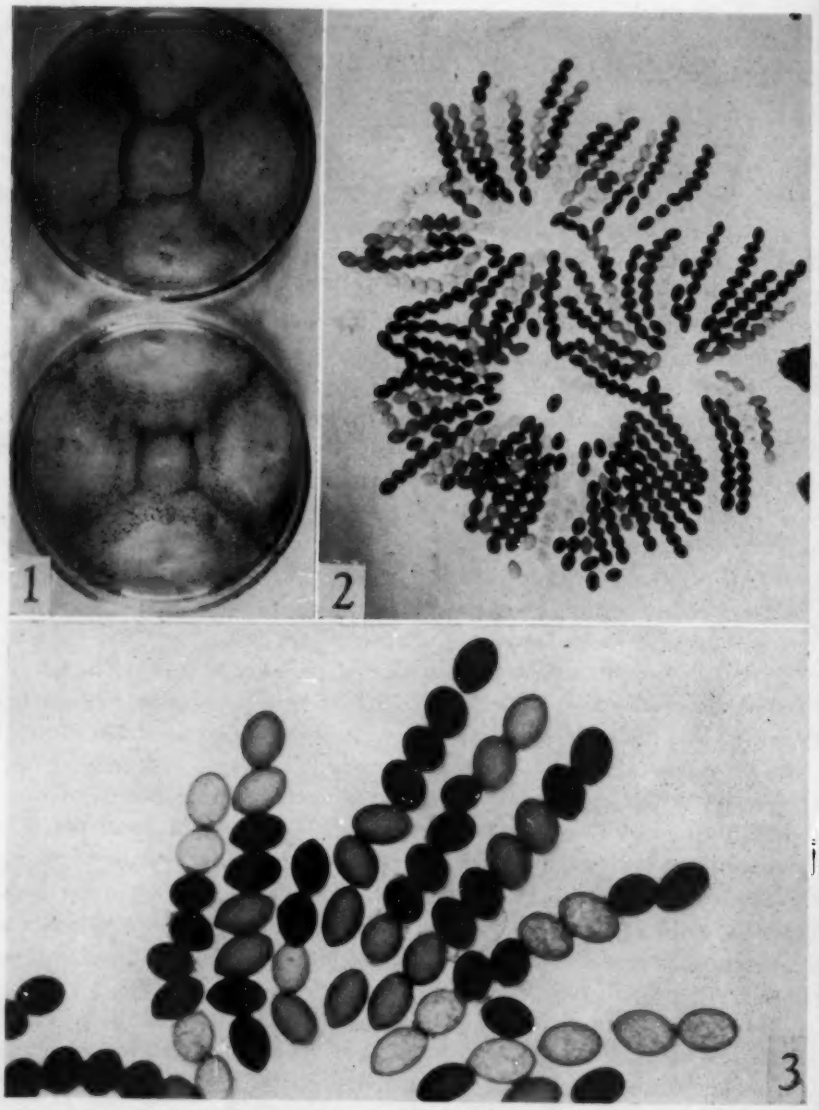
In a recent review (Olive 1953) and a later note (Olive 1954) the writer has called attention to the evidence, definite though not yet extensive as to numbers of species, that cross-karyogamy and segregation occur in homothallic fugi. The prediction was made that these phenomena would prove to be common in homothallic fungi in general.

In order to test this hypothesis further the writer selected for study the pyrenomycete *Sordaria fimicola* Ces. & de Not., which has generally been considered a typical homothallic species. True, Greis (1941/1942) showed that by means of X-ray treatment self-sterile mutants with varying degrees of "maleness" and "femaleness" could be obtained and that certain combinations of these produced fertile perithecia when crossed. He also proved by means of genetic analysis that cross-karyogamy and segregation had occurred in the asci.

In the present study no attempts were made to obtain strains differing in a sexual nature. Cultures were grown in Petri dishes on Difco corn meal agar with 0.1% yeast extract. At the time of perithecial initiation they were subjected to ultra-violet irradiation. When the perithecia had matured they were examined for the presence of mutant ascospores. Normally the fungus produces eight very dark ascospores in a single row in each ascus. Some of the perithecia from the irradiated cultures were found to contain asci with four normal dark and four mutant light ascospores. These spores were plated out and attempts were made to isolate the mutant type. Good ascospore germination was obtained by the acetate method described by Bretzloff (1951). A dozen or more single-spore isolates were secured which produced only asci with light-colored ascospores. The mutant cultures were all stable, of normal growth, and fully self-fertile.

Single-spore mutant cultures were mated with the wild-type culture, the latter being inoculated onto the center of each dish with four mutant cultures surrounding it. Vague or distinct lines of perithecia formed where the mutant cultures came into contact with the wild-type (fig. 1). These lines also formed between mutant cultures. When perithecia from lines formed between mutant and wild-type cultures were examined, they were found to be of three types: (1) pure wild-type, (2) pure mutant, and (3) crossed. The frequency of crossed perithecia was usually quite high. Generally all asci within the same perithecium are of the same genotype, but occasionally segregant and homozygous wild-type or segregant and homozygous mutant asci occur mixed in the same perithecium (fig. 2).

[THE BULLETIN for January–February, 1954 (80: 1–94) was issued February 18, 1954]



FIGS. 1–3. *Sordaria fimicola.* FIG. 1. Mated wild-type (in center) and mutant cultures, ×2/5. FIG. 2. Cluster of asci from crossed perithecium, showing mixture of homozygous wild-type and heterozygous asci, ×150. FIG. 3. Asci showing first- and second-division segregation for the spore color factor, ×620.

The scarcity or absence of homozygous asci in most crossed perithecia indicates that once the mutant and wild-type nuclei are brought together in the same perithecium, the forces of attraction are greater between them than between nuclei of the same genotype. Such a condition, if generally

operative in homothallic fungi, would insure greater variation and would undoubtedly be of great survival value.

A preliminary examination of asci from crossed perithecia has revealed that the spore-color factor segregates two-thirds of the time in the second division, one-third of the time in the first division (figs. 2 and 3). This means that the locus is at least 50 crossover units from the centromere. Further studies are being made to determine whether there is any evidence of preferential cross-karyogamy between any of the normal and mutant isolates. One interesting feature of such a mutant as this is that it can be used to identify crossed perithecia in genetic experiments involving biochemical mutations and other factors that cannot be analyzed in the ascus. Such a mutant might also prove useful in checking the identity of other isolates suspected of being the same species.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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STUDIES ON STREPTOMYCES SCABIES
II. STIMULATION OF MELANIN PRODUCTION BY AMINO ACIDS[1]

JOHN P. HOLLIS[2]

The importance of the tyrosine-tyrosinase reaction in the production of melanic pigments in living organisms is well recognized (Skinner 1939, Harrison 1948, Sussman 1949). This report calls attention to the effects of certain amino acids in stimulating melanin formation by the chromogenic actinomycete, *Streptomyces scabies*. Additional data are presented to show correlations between melanin production and growth and certain features of the reaction in shake cultures.

Materials and methods. Of enrichment substances reported to stimulate melanin production (Hollis 1952b) acid casein hydrolysate (ACH) was selected for detailed study. With the exception of serine and hydroxyglutamic acid, all amino acids known to be present in ACH (Frohring 1950) were tested by single additions to the minimal medium. Only L-isomers of the amino acids were used although limited tests had shown that DL-isomers were effective also.

The minimal synthetic medium used in all experiments contained in g/1: KNO_3 1.52, K_2HPO_4 1.20, $MgSO_4 \cdot 7H_2O$ 0.49, $CaCO_3$ 0.02, Glucose (anhydr.) 8.00. One ml. of a salts mixture was added to yield 0.01 microgram/ml. concentrations of Fe, Zn, Mn, Cu, Mo, Co, and Ga. L-tyrosine was added to the minimal medium at the rate of 1.00 gm. per liter in all experiments involving production of melanin. Growth studies were conducted with the minimal medium above except where the effect of tyrosine was tested. Final pH of the minimal medium with amino acid adjuvants was 7.1 and with added yeast extract 6.9.

Duplicate plates of this medium, solidified with agar and containing concentrations of individual acids equivalent in molarity to 0.1 per cent glycine, were smeared with 0.05 ml. of a filtered spore suspension of *S. scabies* #25 (Hooker 1949) and kept at room temperature. The first appearance of melanin in the supplemented plates in number of hours prior to its appearance in control plates was taken as the index of melanin-stimulation.

In order to determine whether the stimulatory effect of ACH could be attributed in part to the presence of peptides, this substance was separated by two methods (Fromageot, Justisz and Lederer 1948, Winters and Kunin

[1] This investigation was supported by grants from the Research Council, University of Missouri.

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1949) into basic, acidic, aromatic, and neutral non-aromatic fractions. Since mixtures of amino acids simulating the fractions proved to be fully equivalent to the fractions in melanin-stimulating ability, any marked effect by peptidic substances in ACH appeared to be excluded.

Shake culture experiments were conducted with 500 ml. flasks containing 100 ml. of the minimal medium suplemented with 0.1 per cent yeast extract. The flasks were mounted upon a reciprocating shaker having a speed of 84 cycles per minute and a stroke length of 11.5 cm. Glucose was determined by the method of Somogyi (1945) and tyrosine by the 1, 2 nitroso-

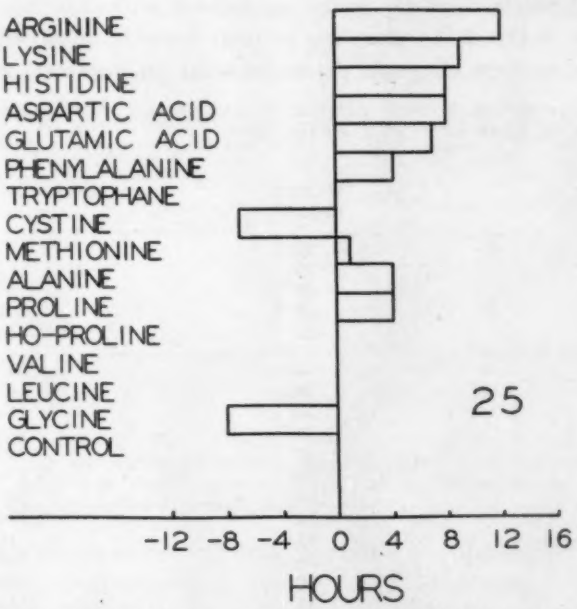

FIG. 1. Melanin-stimulation pattern of *S. scabies* #25, with supplements of individual amino acids (L-isomers), illustrating the appearance of melanin in number of hours (+) before and (−) after appearance in control plates.

naphthol method of Gerngross, Voss and Herfelt (1933) described by Block (1938). After removal of the mycelium, melanin was precipitated twice from each 100 ml. culture by serial additions of 1.0 ml. 10% $FeCl_3$. The reaction of the culture fluid prior to each precipitation was adjusted to pH 6.9. The mycelium and crude melanin were collected individually on tared filter paper, washed with distilled water and dried at 102° C. Although the mycelium was blackened by the presence of melanin[3], its extraction was not attempted. $FeCl_3$ precipitable material from the sterile medium was treated in the same manner as the crude melanin samples and its dry

[3] Melanin retained by the mycelium is called cellular melanin to distinguish it from melanin excreted into the culture fluid.

weight subtracted from weights of total precipitable material at 1, 2, 3 and 4 days to yield weight of melanin by difference. All crude melanin samples were ashed to ascertain the proportion of melanin and impurities present. Nitrogen determinations (Lindner 1944) of the crude melanin samples yielded the following percentages by days: 0–6.59, 1–7.17, 2–8.17, 3–9.21, 4–10.76. Recent work on the spectrochemistry of dopa melanin (Mason 1948, Mason and Wright 1949) indicates it may be a polymer of indole-5, 6-quinone, (N content 9.79 per cent). Assuming a progressive elimination of $FeCl_3$ precipitable impurities up to the third day, the above figures for the third and fourth days are in fair agreement with this theoretical value.

All values in the data, excepting cellular tyrosine plus cellular melanin represent the average of single determinations on duplicate cultures.

TABLE 1. *Comparison between melanin stimulation and growth acceleration properties of amino acids (L-isomers) in S. scabies #25.*

Amino acid	Melanin (hours)	Generation time* (minutes)
Arginine	22	57
Lysine	25	73
Histidine	26	56
Aspartic acid	26	53
Glutamic acid	27	55
Tyrosine	—	340
Phenylalanine	30	142
Tryptophane	34	150
Cystine	41	145
Methionine	33	140
Alanine	30	160
Proline	30	100
HO-proline	34	154
Valine	34	210
Leucine	34	124
Glycine	42	172
Control	34	147

* Generation time in microorganisms may be defined as the average interval between successive cell divisions in a culture.

Experimental results. The melanin-stimulation pattern of *S. scabies* #25, which is a composite of four successive experiments for all amino acids tested, (fig. 1) shows that arginine, lysine, histidine, aspartic acid and glutamic acid are markedly stimulatory. Phenylalanine, alanine and proline are minor stimulants while cystine and glycine are inhibitory. Additional tests indicated that less than 1 part in 10,000 of arginine is required for measurable stimulation of melanin production but the other acids must be present in higher concentrations in an inverse proportion to their lesser activity.

A correlation between growth and melanin production, as occurring simultaneously in culture, was reported previously (Hollis 1952b). In table

1 the correlation is broken down in terms of the individual amino acids. The time in hours required for appearance of melanin is compared with the generation time of growth in minutes on the same medium free of tyrosine. The method of determining generation time has been reported (Hollis 1952a). Growth and melanin-stimulating properties of the basic and acidic acids are correlated. Tyrosine is markedly inhibitory to growth. Of interest is the absence of correlations betwen growth and melanin production with cystine, proline and valine. The values representing melanin production and generation time for the major stimulatory acids differ greatly from control values but the small differences among the minor acids are of questionable validity.

Analysis of the data in table 2 reveals several characteristics of the

TABLE 2. *The production of melanin from tyrosine by S. scabies #25 in shake cultures and its relation to other cultural variables (milligrams in 100 ml culture).*

	Incubation (days)				
	0	1	2	3	4
pH	6.90	6.90	7.07	7.49	7.90
Mycelium	0	12.6	69.7	129.4	186.7
Melanin	0	3.8	5.8	10.1	10.8
Glucose	800	793.04	706.16	414.16	176.56
Tyrosine (medium)	100	80	66	25	17
Tyrosine + melanin (cellular)*	0	16.2	38.2	64.9	72.2

* Cellular tyrosine + cellular melanin was calculated as difference between tyrosine utilized and melanin produced, without correcting for difference in molecular weights between tyrosine and indole-5, 6-quinone.

melanin reaction in shake cultures. Tyrosine is utilized preferentially, with glucose concentration falling rapidly only after about one-half of the tyrosine has been metabolized. The percentage conversion of tyrosine to melanin decreases with time, being respectively: 19.0, 13.2, 13.5 and 11.8 as the tyrosine level drops from 100 to 17 per cent of initial concentration, indicating that melanin production is a function of tyrosine concentration. Both melanin production and cellular tyrosine plus melanin are related in a linear fashion to tyrosine utilization throughout the four day period.

The above facts plus the recorded inhibitory effect of tyrosine on growth in the absence of stimulatory amino acids (table 1) indicate that the conversion of tyrosine to melanin can be interpreted as an overflow or shunt of tyrosine derivatives from other pathways.

There is further evidence on this point. Growth is a direct function of glucose concentration but is related inversely to tyrosine in the medium and accelerates with its progressive removal via the melanin shunt.

Discussion. It would be of interest to determine more exactly the rela-

tive rates of tyrosine and glucose utilization, particularly as regards conversion of tyrosine to melanin. The possibility exists that oxidation of tyrosine may serve as a source of respiratory energy for the organism prior to its more rapid utilization of glucose. Alternatively, the melanin shunt may be only a fortuitous mechanism for elimination of inhibitive tyrosine. Determinations of cellular tyrosine and melanin should aid in resolving this question.

These experiments were performed under the assumption that the conversion of tyrosine to melanic pigment is induced by tyrosinase via a well-known pathway (Mason 1948, Mason and Wright 1949). Although certain elements of the data may justify this assumption tentatively, final proof of its validity must await studies with tyrosinase extracted from the organism.

ACKNOWLEDGMENTS

The author is indebted to W. J. Hooker for the culture of *S. scabies;* to J. Levitt and E. M. Palmquist for criticism of the manuscript and especially to J. Levitt for valuable suggestions and the use of his laboratory.

SUMMARY

1. The amino acids present in acid hydrolyzed casein, with the exceptions of L-hydroxyglutamic acid and L-serine but including L-tryptophan, were tested for biological activity toward *Streptomyces scabies.*

2. Melanin production and growth of *S. scabies* in a synthetic medium containing L-tyrosine were stimulated by single additions of arginine, lysine, histidine, aspartic acid and glutamic acid, (L-isomers).

3. Growth of the organism was inhibited by tyrosine, when this was the only amino acid present, but with additions of stimulatory amino acids, singly or in combinations, growth increased progressively as tyrosine was utilized.

4. In shake cultures the maximum yield of exogenous melanin (under defined conditions and assuming there was no change in molecular weight in the conversion of tyrosine to melanin) was 13.01 per cent of the tyrosine metabolized.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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SHATTERING IN LETTUCE—ITS INHERITANCE AND BIOLOGICAL SIGNIFICANCE

THOMAS W. WHITAKER AND GILBERT D. MCCOLLUM[1]

In seed plants numerous devices have developed for disseminating the mature seed or fruit effectively. In many of the Compositae dissemination is accomplished through modification of the pappus, which acts as a parachute or a grappling organ. Thus modified the pappus functions in the dissemination of the achenes by wind, water, or animals. In *Lactuca serriola* L. and

FIG. 1. Mature capitulum of lettuce, illustrating the shattering character caused by reflexing or shiveling of the involucral bracts at the time the achenes mature.

[1] We are indebted to G. A. Sanderson, Agricultural Aid for the photographs used to illustrate the text.

L. sativa L. dissemination can take place only if the involucral bracts which surround the capitulum (head) are reflexed at maturity exposing the achenes to the action of the elements. At maturity, when the involucral bracts become reflexed, the achenes are exposed, and any slight disturbance will detach them (fig. 1). When this happens, the pappus (acting as a parachute) comes into action, and the achenes may fall to earth at a considerable

FIG. 2. Mature capitula of lettuce, illustrating the non-shattering character. The involucre maintains its cupped shape preventing dissemination of the achenes.

distance from the mother plant depending upon the height of the plant and the strength of the air currents. The senior author has examined numerous colonies of *L. serriola* over a wide area in California and has found only plants in which the involucral bracts were reflexed at the time the achenes were mature. The opposite condition generally occurs in all cultivated va-

[2] An exception is the loose leaf variety, Australian. R. C. Thompson, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, informs us that there is some degree of shattering in this variety.

rieties[2] of lettuce observed by the authors. In most cultivated lettuce the over-lapping bracts are formed into a goblet-shaped involucre that completely surrounds the cluster of achenes at maturity (fig. 2). Thus the achenes are retained within the involucre even though the beak and pappus may be broken off. They cannot be dislodged except by winds, or a sharp, jarring of the plant.

Non-shattering is obviously a very desirable character in cultivated lettuce. It permits comparatively wide latitude in the time of harvest, and assures the seedsman that the mature seed will not fall to earth at the slightest movement of unpredictable air currents. On the other hand, the shattering character in the weedy *Lactuca serriola* may actually have selection value since it insures widespread dissemination of the achenes with a corresponding increase of the chance that they will find favorable ecological niches where they will be able to germinate and reproduce themselves.

In our studies of the biology of the lettuce plant it was considered important to have some information about the inheritance of shattering. Likewise, the relation of this character to other characters better known from genetic studies invites attention.

Observations. The so-called wild lettuce, *Lactuca serriola*, is fully compatible in crosses with cultivated varieties (*L. sativa*); there is little detectable reduction of fertility in F_1 or succeeding generations. Wild lettuce and all cultivated varieties so far examined have 9 pairs of chromosomes.

As a source of the shattering character, the senior author used seed from two individual plants of *L. serriola*, collected in a barley stubble field near Lafayette, California. These plants appeared to be identical except that one had lobed leaves, and the other unlobed. Individuals of the progenies from these plants were used in crosses with two varieties of commercial lettuce, Imperial 615 and White Paris Cos. Imperial 615 is a crisp head type suitable for winter and early spring culture (Bohn and Whitaker 1951). White Paris Cos is a romaine or cos type distinguished by having elongated heads, stiff leaves, and upright habit of growth. The junior author used Prize Head, a variety of leaf lettuce with red-pigmented leaves, and two types of *L. serriola;* the typical form with lobed leaves was collected at Pullman, Washington, and another form, with only partly lobed leaves having unlobed, narrowly tapered tips near Grangeville, Idaho. The crosses are indicated below; those involving Prize Head were reciprocal matings.

Imperial 615 × *L. serriola* (lobed)
White Paris Cos × *L. serriola* (unlobed)
L. serriola (unlobed) × Imperial 615
Prize Head × *L. serriola* (Pullman)
Prize Head × *L. serriola* (Idaho)

The shattering character can be easily scored if the head has reached maturity. Otherwise, the involucral bracts may not be reflexed and the plant is likely to be mis-classified as non-shattering.

In the F_1 all the plants were definitely of the shattering type. Seven F_2 families were grown; two from Imperial 615 × *L. serriola*, two from White Paris Cos × *L. serriola*, and one each from *L. serriola* × Imperial 615, Prize Head × *L. serriola* (Pullman), and Prize Head × *L. serriola* (Idaho). The results are recorded in Table 1. All families segregated normally, if it is assumed that non-shattering depends upon a single recessive gene, except family 21645 for which P barely exceeds the 5% point. However, chi-square for the pooled data, sum of chi-squares, and heterogeneity are non-significant. These statistical tests suggest that a single gene hypothesis is valid. The letter b is suggested as a symbol for this gene.

TABLE 1. *Segregation in F_2 for shattering and non-shattering.*

Family	Shattering	Non-shattering	Total	$\chi^2_{3:1}$
21642	49	20	69	0.58
21643	28	10	38	0.04
21645	48	26	74	4.05*
21646	66	18	84	0.57
21647	31	16	47	2.05
"X Pullman"	337	133	470	2.73
"X Idaho"	139	44	183	0.09
Total	698	267	965	10.11

Pooled $\chi^2 = 3.66$
Heterogeneity $\chi^2 = 6.45$

* χ^2 significant at 5% level

Linkage. Since there are no techniques for achieving close control of emasculation in lettuce (Bohn and Whitaker 1951), it is not expedient to use the backcross method for linkage tests. Segregations obtained in F_2 and F_3 progenies are the most practical means for the detection and estimation of linkage intensities. By use of the partitioning of chi-square method of analysis developed by Mather (1938), the shattering gene was tested for linkage with the genes for achene color and leaf lobing.

The inheritance of achene color was worked out by Thompson (1938, 1943). Black is dominant over white and depends upon a single gene for its expression. *Lactuca serriola* in the matings used has black achenes, while the cultivated varieties used have white achenes. In tests for linkage between shattering and achene color, F_2 segregations from 7 families, with a total of 964 individuals, were used. The calculations give good independent segregation ratios for both genes with no indication of linkage (Table 2). Progenies from 5 doubly heterozygous individuals were tested in F_3 for

TABLE 2. *Segregation in F_2 for shattering and achene color.*

Family	BW	Bw	bW	bw	χ^2 9:3:3:1
21642	35	14	14	6	1.21
21643	22	6	5	5	3.73
21645	39	9	20	6	5.00
21646	44	22	15	3	3.70
21647	23	8	14	2	3.87
"X Pullman"	264	94	73	39	6.14
"X Idaho"	101	35	37	9	0.78
Total	528	188	178	70	24.43

Pooled $\chi^2 = 2.28$

Heterogeneity $\chi^2 = 22.15$

Linkage tests	χ^2
Segregation for B, b	2.89
Segregation for W, w	1.60
Joint segregation	0.41

linkage between the shattering and achene color; a total of 207 plants was scored with the same results as noted for the F_2 population.

In lettuce, certain types of leaf lobing segregate in monogenic fashion (Whitaker 1950).[3] In tests for linkage between shattering and lobing in the F_2, 566 plants were scored (Table 3). In F_3, seven progenies from plants

TABLE 3. *Segregation in F_2 for shattering and leaf lobing.*

Family	BU	Bu	bU	bu	χ^2 9:3:3:1
21642 and 21643	59	18	18	12	4.67
"X Pullman"	234	96	95	34	5.33
Total	293	114	113	46	10.00

Pooled $\chi^2 = 6.24$

Heterogeneity $\chi^2 = 3.76$

Linkage tests	χ^2
Segregation for B, b	2.89
Segregation for U, u	3.22
Joint segregation	0.20

heterozygous for both genes, a total of 395 individuals, were scored. The calculations for both F_2 and F_3 families were negative for linkage between the two genes.

Discussion. The shattering condition, while simple genetically, is evidently of considerable importance to the species for its existence outside of cultivation. It is probably one of the factors enabling the wild form (*Lactuca serriola*) to become a successful weed of roadsides, fence rows, and waste places in this country and Europe. If, as Ownbey's (1949) observations indicate, there is a continuous introgression of cultivated lettuce into

[3] The type of lobing in the Idaho *L. serriola* did not segregate as a monogenic character, and tests for linkage with shattering were not attempted.

its non-cultivated prototype, our observations suggest that the non-shattering recessives are eliminated very quickly under non-cultivated conditions. This would be expected, because without dissemination, competition between the young seedlings for moisture, light and nutrients would be confined to a very small space immediately around the mother plant. Incidently, it seems unlikely that the recessive condition had survival value prior to the domestication of lettuce by man.

The loss of natural adaptations for the dissemination of seeds in cultigens is not confined to lettuce. Wulff (1943), in commenting on this phenomenon cites a number of examples. For instance in cereals, the character of many jointed spikes (brittle spikes) is common among wild species, but is absent or nearly so in the closely related cultivated species. Among the Umbelliferae bipartite fruits are the rule in the wild species, while this character is suppressed or less pronounced in the corresponding cultivated species. Fruits of cultivated flax and opium poppy do not dehisce when ripe, while those of wild flax, *Linum crepitans* Dum., and wild poppy, *Papaver Rhoeas* L. are dehiscent when mature. A similar phenomenon is taking place in castor beans, *Ricinus communis* L. Under the guidance of plant breeders, shattering types are being replaced by shatter-resistant ones (Domingo 1953).

SUMMARY

1. The shattering character in lettuce is described and shown to be the expression of a single dominant gene. In general, it is found only in wild lettuce, *Lactuca serriola*. The recessive allele (non-shattering) is for the most part restricted to cultivated varieties. Shattering is not linked with either achene color or leaf lobing.

2. Indirect evidence suggests that shattering is of considerable importance to the species under non-cultivated conditions. On the contrary, under cultivated conditions, non-shattering is the favored type, and shattering becomes detrimental. It is pointed out that loss of natural adaptations for the dissemination of seeds has occurred in many cultivated plants.

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METHOD OF CULTURE AND GROWTH OF MAIZE ENDOSPERM IN VITRO[1]

ELIZABETH PIECZUR STERNHEIMER

In 1949, LaRue reported the first culture of maize endosperm tissue having unlimited growth *in vitro*. A preliminary investigation of its effect on embryo growth has been made which showed that its function as a nutritive endosperm tissue had been retained after *in vitro* culture (Pieczur 1952). This paper is a report on the establishment of new stocks and the continued growth of the tissue *in vitro* up to the present time.

To initiate a new culture, the endosperm of the corn grain was excised under sterile conditions ten to twelve days after pollination. After four to eight weeks on the appropriate medium described below, there appeared callus-like outgrowths of the excised endosperm tissue. When a variety of maize which possesses an anthocyanin-pigmented aleurone was cultured, the callus-like growths contained areas which had the purple pigment and areas which did not have it. When the aleurone layer was colorless, no such differences could be distinguished. Growth appears to be due to a continuation of cell division in the aleurone and underlying layers of the endosperm. The cells of these layers are dividing at the 10–12 day stage in the normally developing endosperm *in vivo* (Brink and Cooper 1947). When freed from the restraining outer coats of the corn grain, it would seem that these cells can achieve the capacity of unlimited growth *in vitro*.

The callus-like growths which are a faint yellow in color, or deep purple from a purple aleurone, were removed and transferred to fresh medium where growth continued. After several months, during which time, the tissues were subdivided and transferred every few weeks, the growth rate became accelerated. A small fragment would in three weeks become a large mass. Its weight would increase three to eighteen fold in this period depending upon the strain and the growing conditions.

Growth of the endosperm tissue from the time of excision requires a medium containing tomato juice. The factors necessary for growth which are present in the juice are as yet unknown. The basic medium employed was White's to which was added 20 per cent by volume clear tomato juice which had been adjusted to pH 6.5 (White 1943). The clear tomato juice was obtained by centrifuging the thick juice from sieved canned whole unsalted tomatoes. The medium was autoclaved after the tomato juice had been added. The cultures were grown on agar slants in the dark at 25° C.

[1] Studies carried out under an AEC Predoctoral Fellowship. I wish to thank Dr. W. R. Singleton for the corn material he generously provided.

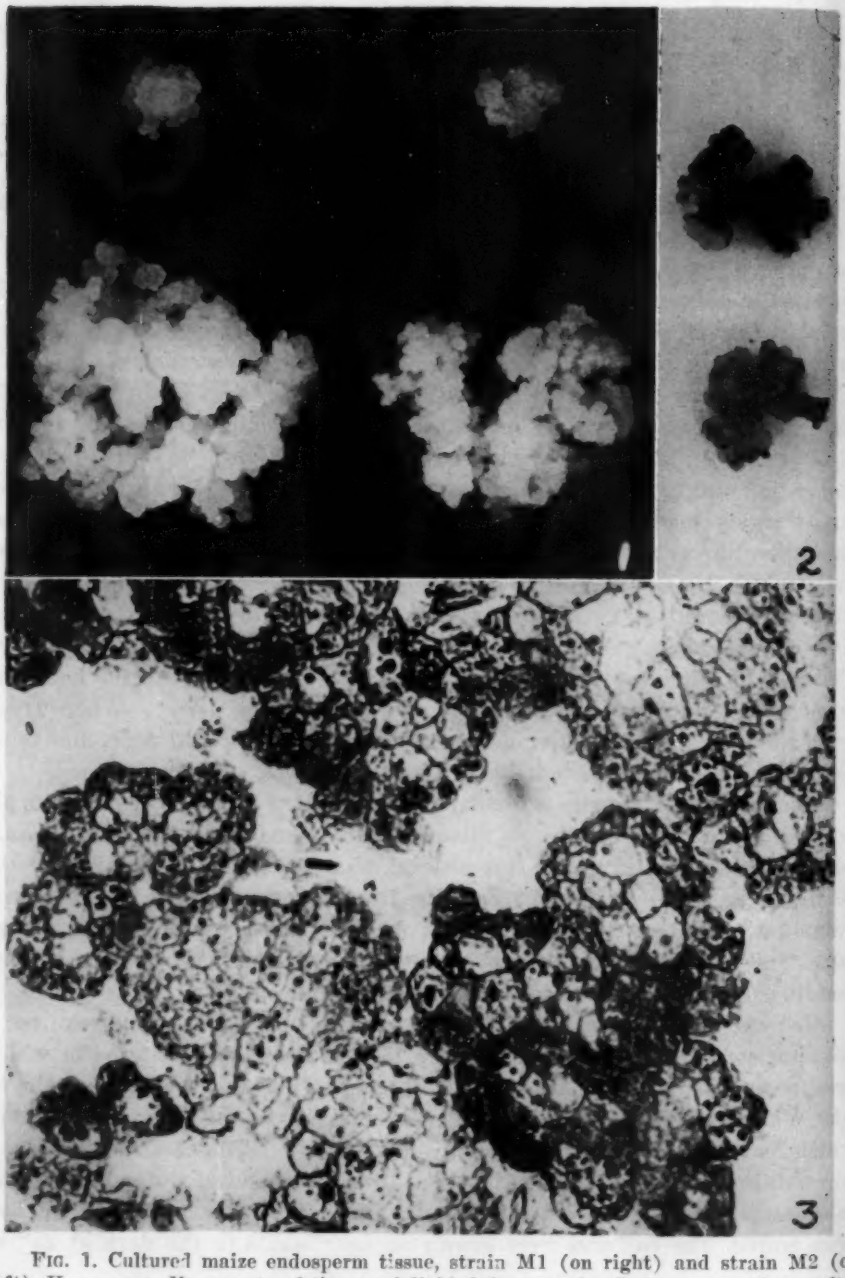

FIG. 1. Cultured maize endosperm tissue, strain M1 (on right) and strain M2 (on left). Upper row: Fragments of tissue subdivided for transfer. Bottom row: tissue after three weeks of growth. ×2. FIG. 2. Cultured maize endosperm tissue, strain M3. ×2. FIG. 3. Portion of stained section of cultured maize endosperm tissue, strain M1. ×80.

After the growth rate had become accelerated, stock cultures were transferred every three weeks.

Many varieties of waxy (*wx*), starchy (*Su*) and sugary (*su*) endosperm types of maize were placed in culture from stocks grown by Dr. W. R. Singleton at Brookhaven National Laboratory in the summer of 1951. None of the waxy and starchy endosperms grew under the conditions described above. Only the sugary types, so far, have been cultured successfully. Several new strains were established, the most interesting of which is being maintained as a stock culture. This strain, designated as M3, was obtained from the sweet corn variety Black Mexican which possesses an anthocyanin-pigmented aleurone layer in the endosperm. A pigmented and non-pigmented set of stock cultures of M3 are now in the twentieth transfer. Strain M1 which was originally placed in culture by LaRue, was obtained from him in 1949. It is now in its fifty-seventh transfer. Strain M2 was established by the author in the summer of 1950 from a sweet corn hybrid variety, Marcross 6.13, originally obtained under the name of Our Choice. It is in its thirty-sixth transfer. Figures 1 and 2 show cultures of M1, M2 and M3 tissue. In all strains, the mass of tissue is very friable. It consists of small meristematic cells and parenchymatous cells of varying sizes. Figure 3 is a photograph of a stained section of M1 tissue.

LaRue found the excised endosperms, in a few cases, to have regenerated roots and stems (LaRue 1947). Such regeneration has not been observed in any of the cultures of the various varieties of maize grown by the author.

SUMMARY

1. The method of culture of maize endosperm tissue *in vitro* is described.

2. A report is given of the growth of three strains of the cultured maize endosperm tissue which have been maintained to the present time.

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MITOCHONDRIAL RESISTANCE TO THE ACTION OF CHEMICAL CARCINOGENS

DON RITCHIE[1]

That mitochondria are involved in some types of cancer is indicated by investigations which, through the past several years, have been building up evidence of the implication of cytoplasmic particles in abnormal growth. DuBuy, Woods, Burk, and Lackey (1949) found that centrifugally isolated dark granules from several mouse melanomas were associated with some of the enzymes characteristic of mitochondria: cytochrome oxidase, succinic dehydrogenase, cytochrome C. These demonstrations, plus others, led them to suggest that the cytoplasmic granules in the melanomas studied represented mitochondria. Hogeboom and Schneider (1951) compared mitochondria from normal mouse liver with those from a hepatoma. When investigated in the analytical ultracentrifuge, the mitochondria from the two sources differed in several ways in their component proteins. The most striking difference was that when the mitochondria were disintegrated by sonic vibration, they possessed four protein components, but the mitochondria from the hepatoma possessed only three.

The problem of the relation of mitochondria to cancers has been hopefully attacked by the use of chemical carcinogens. Goerner (1938) showed that injection of dibenzanthracene caused reduction or abolition (depending on the dose) of the vitamin A content of the mitochondria in rabbit liver. The actual dosage was not specified, animals being killed "when the number of injections and the elapsed time appeared suitable."

Levine (1950), seeking information on cancerous growths in plants treated with carcinogens, showed, in a series of investigations, that various species reacted differently. Carrot tissue grown *in vitro* in the presence of carcinogens showed atypical cells, but stem segments of tobacco and sunflower did not. These plants were exposed to various concentrations of 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 20-methylcholanthrene in slow gradients from 0.1 mg. per cent to 5.0 mg. per cent. In sunflower, no reaction that could be construed as tumor or malignant growth appeared. In fact, the carcinogens enhanced the growth of the roots and sustained the growth of the tissue mass. Growth of complete plantlets of tobacco from tissue masses was not directly attributed to the carcinogens, "but the evidence is clear that these chemicals did not interfere with the tendency to bud development."

In all of the investigations cited, conclusions depended on maceration or

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chemical fixation of the cells, with the pitfalls attendant upon these methods. The sources of error inherent in techniques which depend upon destruction of the cell have been discussed by Chambers (1943) and Weier and Stocking (1952), and the difficulties of studying mitochondria in fixed and stained sections have been described by Newcomer (1946).

Environmental changes are known to produce variation in mitochondrial size, numbers, shape, motility, refractivity, staining reaction, physical strength, internal structure, and orientation within the cell. The experiments reported on in the present paper were designed to find whether detectable modifications in mitochondria in a living plant could be produced by the incorporation of carcinogenic chemicals in the nutrient media. For such work, it is desirable to avoid the difficulties mentioned in the preceding paragraph. The advantages of an aquatic fungus for mitochondrial investigations have been pointed out elsewhere (Ritchie & Hazeltine 1953, Ritchie 1953). The weakness is that which is inherent in a morphological technique: subtle changes may be overlooked.

Material and methods. The plant employed was *Allomyces javanicus* Kniep. Pure cultures of the fungus were grown on a basal agar medium, at pH 7.0, containing 2% dextrose, 1% maltose, 1% yeast extract, Na_2HPO_4, KH_2PO_4, and $MgSO_4$. The carcinogens employed were 20-methylcholanthrene and 3,4-benzpyrene. A sample of 1,2,5,6-dibenzanthracene which could not be dissolved in benzene at room temperature was not followed through.

The medium containing the carcinogens was prepared by dissolving them in benzene and making dilutions so that each desired quantity of solute could be contained in 0.5 ml. of solution. The 0.5 ml. quantities were added to 1.5 ml. samples of solution containing the inorganic salts, mixed thoroughly, and autoclaved at 15–18 pounds for 20 minutes to drive off the benzene. The mixture of carcinogen and salts was then added to an equal quantity of liquid containing the organic constituents of the basal medium which had been sterilized separately to avoid reaction between the phosphates and the sugars during autoclaving. A 1 ml. portion of the final product was poured as a thin film over the surface of a sterile microscope slide which was supported in a Petri dish containing sufficient water to retard desiccation of the agar. Media were made with the following concentrations of constituents.

All experiments were set up in triplicate, and hyphal slips were planted on the agar surfaces. Cultures were grown for five days in the 30° C. incubator, where they were covered by loosely fitting metal cylinders to help retard drying.

When results were to be recorded, the surface of the agar containing mycelium was covered gently with a cover glass, and observations were made by means of the phase contrast microscope. That this procedure does not

harm the plants is indicated by the fact that cells so treated differ in no visible respects from those grown in water cultures and examined in water.

BASAL AGAR MEDIUM PLUS

No.	Benzene	Mg. methylcholanthrene/ 100 ml. medium	Mg. benzpyrene/ 100 ml. medium
1	0.0	0	0
2	0.5	0	0
3	0.5	1.0	1.0
4	0.5	5.0	5.0
5	0.5	10.0	10.0
6	0.5	13.3	13.3
7	0.5	16.7	16.7
8	0.5	20.0	20.0

Observations and results. Some of the concentrations of carcinogens used in these experiments were very high in comparison with previously reported dosages. They were tried because early trials with low quantities produced no effects upon the plants. In fact, even when the concentrations reached 20 mg. per 100 ml. of medium, the mitochondria remained typically long, refractive, and independently active. When mycelia so treated were transferred back to their usual aquatic habitat, they grew in an apparently perfectly normal fashion. As far as the mitochondria are concerned, it seems that they are strongly resistant to the action of these particular carcinogens within the concentrations tested. It is not known whether the large, insoluble molecules ever entered into any living cell.

Aside from the mitochondria, however, it was noted that nucleoli, even in the lower concentrations, were sometimes abnormally large. Their size variation was not studied statistically. In the medium containing benzpyrene, the zoosporangia, which are normally terminal and contain from 40 to 60 or even 100 zoospores, were frequently intercalary and much reduced. Many sporangia were no larger in diameter than the hypha which produced them, and contained a single line of spores. In rare instances, as few as two spores were formed in a sporangium. These mycelia nevertheless produced normal plants when transferred to water.

How much effect an extracellular substance which is insoluble in water can have on the intracellular environment is not known. In view of Goerner's (1938) demonstration of the reduction in vitamin A content of tumor mitochondria, there is undoubtedly some effect, since he used careful controls to prove that the reduction was not simply a result of nutrition. Since the male gametes of *Allomyces* contain carotene (Emerson and Fox 1940) and are visibly colored, it will be interesting to determine whether a carcinogen can affect the quantity of carotene which is normally accumulated in these cells. The problem, which will be reported on later, should give further indication of the intracellular influence of chemical carcinogens.

SUMMARY

1. Mycelia of the aquatic Phycomycete *Allomyces javanicus* Kniep were grown in agar media containing a dispersion of the chemical carcinogens, 20-methylcholanthrene and 3,4-benzpyrene, in a series of concentrations from 1 to 20 mg. per 100 ml. of medium.

2. When examined by means of phase contrast microscopy, mitochondria were as large, numerous, active and refractive in very high concentrations of carcinogens as they were in the basal medium alone or basal medium plus benzene.

3. In the benzpyrene media, some instances of greatly reduced zoosporangia were noted, but the spores themselves were apparently not affected.

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TROCHODENDROXYLON BECKII GEN. ET SP. NOV. FROM THE TERTIARY OF OREGON

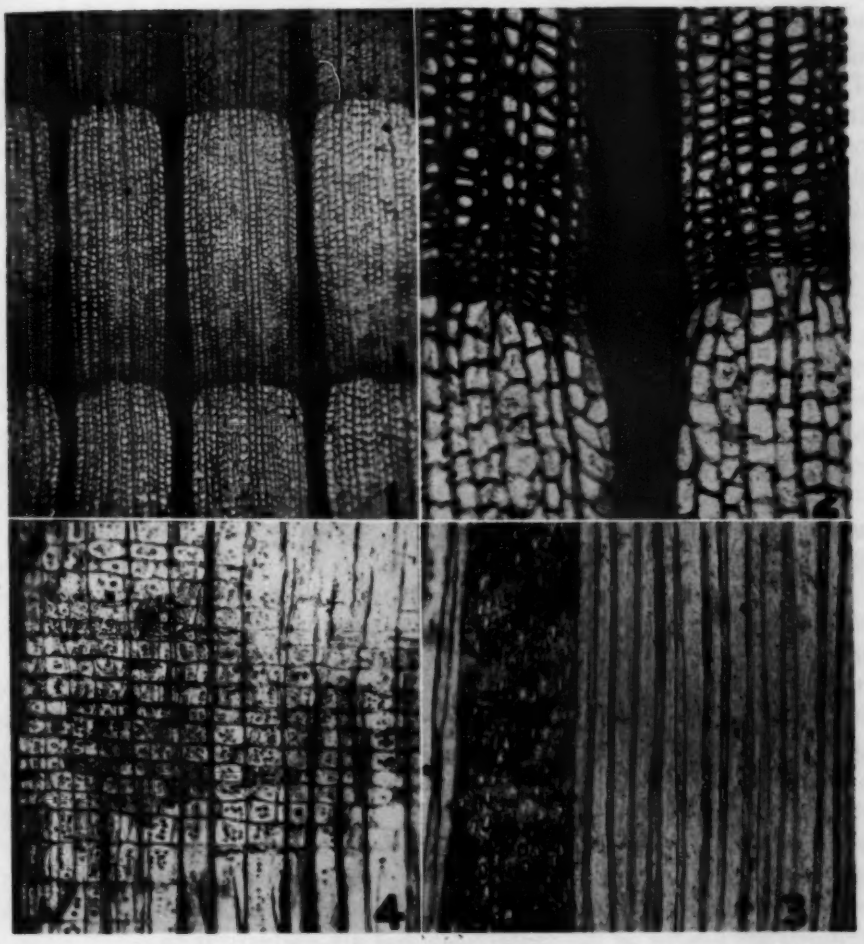
H. L. HERGERT AND H. K. PHINNEY

At present members of the monotypic families Trochodendraceae and Tetracentraceae are confined to Formosa, Japan, southern China and northern Burma. The discovery, however, of fossil vesselless wood characteristic of these families in eastern Greenland, India, and northwestern United States indicates that they had a wide distribution at one time. Beck (1941) described a fossil wood found in western Washington which he placed in the genus *Tetracentron*. He further indicated (1944) that a fossil wood resembling *Trochodendron* was obtained from a site near Sweethome, Oregon, but did not give a description of the wood. The reference of this specimen to a specific genus is questionable since Beck stated he was unable to distinguish between the two genera. Sanborn (1935) assigned to the Trochodendraceae a fossil leaf from the upper Eocene of western Oregon.

In the course of a study of the fossil floras found near Sweethome, Oregon, and in adjacent areas we discovered a vesselless wood among the abundant available silicified specimens from the area. This particular wood has been found in four different localities in Linn County, Oregon. The most productive of these localities lie in an area about one and one-half miles due east of Holly (Oregon State College Locality Nos. 1226, 1227, and 1228). The wood has also been found two miles south of Berlin (Locality No. 1229), and in the Willamette River gravels near Corvallis. In all of the areas save the last, the wood is weathered out of a tuff formation which we assume to be the continental equivalent of the Eugene formation, and therefore middle or upper Oligocene in age. Previously a fossil evergreen cherry leaf (Brown 1950) and a fossilized wood presumed to be *Platanus* sp. (Staples 1950) were recorded from the Sweethome area.

The fossil wood described here is completely silicified. It was sectioned by the usual method for such material. The figured thin sections came from a piece of wood (Locality No. 1227) approximately 12 x 16 x 20 cm. in size. The tree from which the piece was derived is estimated to have had about 160 growth rings and a diameter of about 40 cm. The sections were made at a point representing about 75 years of growth of the tree.

Trochodendroxylon gen. nov. The wood, resembling that of the modern genus *Trochodendron*, is vesselless, angiospermous wood with conspicuous growth rings. The rays are of two types: uniseriate rays and large, multiseriate, heterogeneous rays. The tracheids of the early wood are large and thin-walled, with scalariform bordered pitting. The late wood tracheids are smaller and thick-walled, with small circular bordered pits.



FIGS. 1–4. Photomicrographs taken of thin sections of the wood of *Trochodendroxylon beckii*. FIG. 1. Trans. sect. Prominent multiseriate rays show characteristic variation in width in early and late wood. × 25. FIG. 2. Trans. sect. Size and shape of tracheids in early and late wood. × 100. FIG. 3. Tang. sect. Multiseriate ray and comparatively inconspicuous uniseriate rays. × 100. FIG. 4. Rad. sect. Procumbent cells (center) and erect marginal cells (lower left) of the multiseriate rays. × 100.

Trochodendroxylon Beckii sp. nov.[1] **Transverse section** (figs. 1–2). The annual rings are of variable width. The maximum width is 1.9 mm., the average 1.1 mm. The transition between the early wood and the late wood is abrupt. The early wood tracheids are more or less square, 25 μ in diameter,

[1] Named in honor of Professor George Beck of Ellensburg, Washington, whose efforts have made clear the great paleobotanical significance of fossil woods in elucidating the fossil floras of the Northwest.

with a cell wall 1 to 1.5 μ in thickness. The late wood tracheids are tangentially flattened with walls averaging 2.5 μ in thickness (maximum 6 μ). Two types of rays are present: numerous uniseriate rays separated by from one to three rows (usually two rows) of tracheids, and multiseriate rays which average 12 cells wide (14 cells maximum). At their widest, in the late wood, the multiseriate rays average 190 μ (maximum 260 μ); at their narrowest, in the early wood, the multiseriate rays average 110 μ (maximum 150 μ).

Tangential section (fig. 3). The tracheids are very long with an average length of 4.7 mm. and a maximum length of 6.1 mm. Few pits are found on the tangential walls. The uniseriate rays are 15–20 cells high (up to 1.1 mm. in height), and are composed of vertically elongated cells 55–60 μ high by 5–10 μ wide tangentially. The heterogeneous multiseriate rays average 4.0 mm. in height with a maximum height of over 4.3 mm. (150 cells). The cells of these multiseriate rays as seen in tangential view are of two types: central cells that average 20–25 μ high (maximum 32 μ) by 5–10 μ wide, and marginal cells averaging 35 μ high (minimum 30 μ, maximum 65 μ) by 5–10 μ wide.

Radial section (fig. 4). In the radial section the heterogeneous nature of the multiseriate rays is clarified. The marginal rows, 10–15 cells high, are of upright cells 20–25 μ wide radially. They contain one to two vertical rows of pits to tracheids, usually four to six pits in a row. These pits are 4 μ in diameter and are typically circular, with lenticular to slit-like apertures often exceeding the circular borders, but are transitional to scalariform in the early wood. The central portion of the ray is composed of procumbent cells 100–140 μ wide. The uniseriate ray cells are 25–30 μ wide radially. The early wood tracheids have scalariform pitting while the late wood tracheids have circular bordered pits, averaging 4 μ in diameter, with lenticular to slit-like apertures often exceeding the circular borders. The pits are mostly on the radial walls but not exclusively so.

In longitudinal section it can be seen that the wood parenchyma strands are uniseriate, with anticlinal partitions more or less obliquely oriented. Biseriate parenchyma strands were not observed. No oil cells have been observed.

Discussion. The obvious structural characteristics of this wood, i.e., the heterogeneous, multiseriate rays, the distinct annual rings, and the absence of vessels, clearly limit comparison of it to the modern monotypic genera *Trochodendron* and *Tetracentron*. Baily and Nast (1945) pointed out: "... the woods of the two genera are so similar as to render difficult and uncertain the task of determining to which genus certain fossil woods from significant geological horizons of India, Greenland, and northwestern United States are related. ..." However, these and other authors have

enumerated several criteria for distinguishing between the two genera of living woods, and we have been able to apply some of them to our material.

Tetracentron sinense Oliv. contains a type of wood parenchyma unique in that it is present as biseriate strands. Such strands are absent in *Trochodendron araliioides* Sieb. & Zucc. and in *Trochodendroxylon*. The number of multiseriate rays in the oldest secondary wood of *Tetracentron* is small while such rays are numerous in *Trochodendron* and *Trochodendroxylon*.

As pointed out by McLaughlin (1933) the wood of the Tetracentraceae and the Trochodendraceae show other points of difference. The multiseriate rays in the mature wood are not only wider in *Trochodendron* (240 μ) and *Trochodendroxylon* (150 μ) but also they are much further apart than in *Tetracentron* (width 60 μ).

These characteristics indicate that our wood is very similar to the wood of living *Trochodendron*. The fossil wood differs, however, in the following respects. The multiseriate rays of the fossil wood are considerably higher and show a greater number of cells in width, although they are narrower. Both the average length and the maximum length of tracheids are greater in the fossil than in the living species. Wood parenchyma in the cross section of *Trochodendron* tends toward organization into unseriate lines while in *Trochodendroxylon* the parenchyma is diffuse and only rarely in rows. This difference may be due to the fact that the late wood zone is much narrower in the fossil than in the living species.

Comparison of our fossil wood with previously described fossil, vesselless angiosperms also demonstrates decided differences. For the most part, these other fossils resemble *Tetracentron* rather than *Trochodendron*. *Homoxylon rajmahalense* Sahni (1932) from the Jurassic of India has rays which in height and width are more suggestive of *Tetracentron* (Gupta 1934). *Tetracentronites hartzii* Mathieson (1932) from the Eocene of Greenland is of rather uncertain relationship since the description of the critical diagnostic features is lacking. It appears to have a close affinity with *Tetracentron* by reason of the width of its rays. The figures of Beck's (1941) fossil wood from western Washington indicates *Tetracentron*-like rays and the suggestion of biseriate parenchyma.

We have, therefore, named this fossil wood to distinguish it from these previously described fossil species as well as from the living species. Because the fossil wood differs in some details from that of the living species, and because we have no material to indicate the structure of the other organs of the tree, we have deemed it advisable to follow modern paleobotanical practice and separate the fossil from the living genus. Therefore the form genus *Trochodendroxylon* has been established for *Trochodendron*-like fossil woods.

SUMMARY

1. A number of specimens of a fossil, vesselless, angiospermous wood have been collected near Sweethome, Linn Co., Oregon.

2. The horizon is presumed to be middle or upper Oligocene and a continental equivalent of the Eugene formation.

3. A new form genus of fossil wood, *Trochodendroxylon*, has been erected to contain fossil woods showing affinities to the genus *Trochodendron*.

4. The species **Trochodendroxylon beckii** nov. sp. has been described and figured as the type of this new genus.

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THE ORIGIN AND DEVELOPMENT OF THE DIAPHRAGM AT THE BASE OF THE INTERNODE OF *EPHEDRA CORYI*

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A band of narrow cells elongated in the tranverse direction, which form a plate-like diaphragm through the stem at the base of each internode, is a feature peculiar to the genus *Ephedra* (Coulter and Chamberlain 1917). The development of a diaphragm has been explained to be a result of intercalary growth, as found in stems of *Zea, Narcissus, Equisetum* and *Nitella* (Moldenhawer 1812, Meyer 1832, Hofmeister 1851, Askenasy 1881).

Jacobs (1947) found that the intercalary zone of the gynophore of the peanut (*Arachis hypogaea* L.) resulted from a resumption of the meristematic activity of cells which were apparently stimulated by some phenomenon associated with pollination. The cells forming this zone retained the ability to divide long after the cells of the rest of the internode.

Diaphragms found in the internodes of *Ephedra* differed radically from those found in *Juglans* where they separate the pith into a vertical row of chambers, or those found at the nodes of *Equisetum* (Browne 1939). Stapf (1889) described the origin of the diaphragm of *Ephedra altissima* as a meristem, the cells of which divided very slowly. Ultimately there was a differentiation of all the cells in the internode with the exception of the narrow diaphragm. In this species the zone traversed the entire internode, remaining in a resting condition until it was completely differentiated.

Observations by Graham (1908) were similar to those of Stapf (1889), except that Graham showed that the diaphragm had cut partially through the vascular tissues and the xylem connections were retained by short-reticulate tracheids.

Ephedra coryi is indigenous to the plains of western Texas with a restricted distribution in southeastern New Mexico. Plants used in this investigation were collected from this region to study the development of the internodal diaphragm. The material was kept moist until it was killed and fixed in F.A.A. solution (90 cc ethyl alcohol 70%, 5 cc glacial acetic acid, 5 cc formalin). The samples were dehydrated in tertiary-butyl alcohol, embedded in paraffin, sectioned 10 microns in thickness, stained with safranin and fast green, and mounted permanently in Canada Balsam.

Major changes linked with the formation of the diaphragm in the internode of *Ephedra coryi* appeared to be in the pith. In the early developmental stages the cells of the entire internode tended to be more or less

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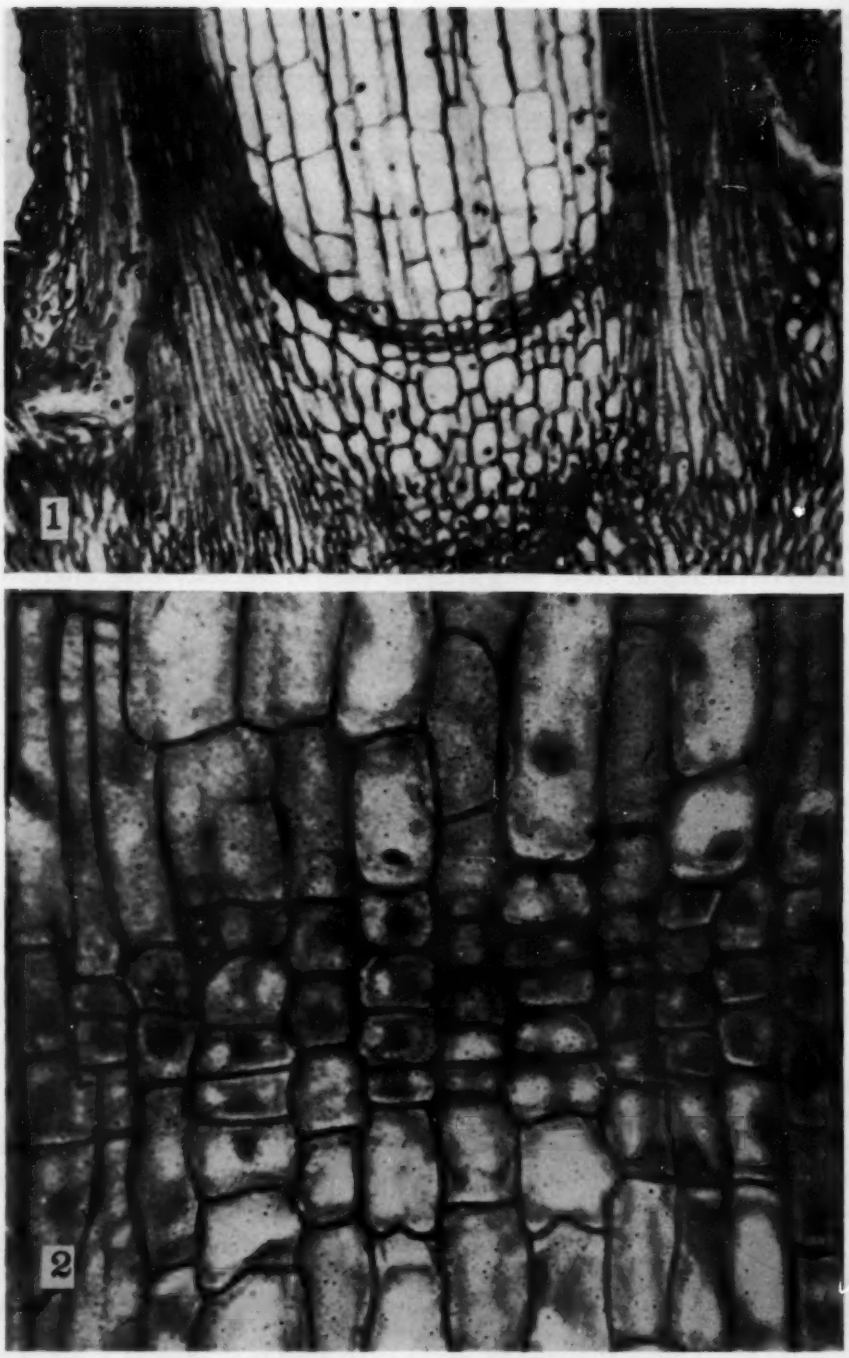

FIG. 1. Longitudinal section of the stem of *Ephedra coryi*, showing the diaphragm cells above the 5th node. ×40. FIG. 2. Longitudinal section of the internode showing the pith region at the level of the diaphragm, with the differentiation of the adjacent cells above the 7th node. ×225.

meristematic. Beginnings of differentiation were evidenced by a slight elongation and vacuolation of the pith cells at the distal end of the internode.

In the region of the apical node, there was no histological evidence of a diaphragm. Very little differentiation had occurred in the entire 2nd or 3rd internode, but in the 4th internode (fig. 1) the cells had increased in size in all dimensions. The location of the diaphragm in relation to the node is visible in this same figure. Differentiation occurred proximally from above, and distally from below, the diaphragm. At the distal end of the internode, the cells were approximately twice their original size, being progressively more meristematic toward the proximal end of the internode.

Cells located between the diaphragm and the node (fig. 2) increased in longitudinal and transverse dimensions. While it is not clear in this photograph, the cell walls ultimately became thicker on cells close to the nodal region situated proximally to each diaphragm. These cells were more completely differentiated and appeared quite mature when contrasted to the highly meristematic condition of the cells forming the diaphragm. The location of the diaphragm was controlled by the amount of differentiated tissue which had developed between it and the most adjacent node. The diaphragm was composed of residual meristematic tissue in the center of the pith. It was from three to five cells in depth and the layer became progressively thicker toward the periphery of the stem where it was as much as eight cells in depth.

In all internodes below the third, the cells continued differentiation and enlargement both proximally and distally. Ultimately, in the ninth and tenth internode all of the cells between the diaphragms and the adjacent nodes were differentiated. A mature diaphragm was usually two to four cells in vertical thickness and these cells retained their original meristematic condition.

SUMMARY

1. Stems of *Ephedra coryi* were collected, dehydrated and permanent longitudinal and transverse sections were mounted for examination.

2. Manner of differentiation of the cells composing the internode of this plant was described to illustrate that an internodal diaphragm composed of meristematic tissue was located chiefly in the pith region of the internode and that it developed as a result of uneven cellular differentiation within that internode.

3. Maturation of cells was shown to have occurred proximally from above and distally from below the location of the diaphragm in the internode.

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A PRELIMINARY STUDY OF THE STAINING OF PLANT CELLS BY TETRAZOLIUM CHLORIDE

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Tetrazolium (2,3,5-triphenyltetrazolium chloride[1] or TTC) and a few related compounds have been of interest to biologists for the last ten years. Smith (1951) has reviewed the subject of tetrazolium in relation to enzymes and staining of plant tissues. The relationships of TTC to certain reducing enzyme systems and the use of TTC in staining living tissues, especially plant embryos in seeds as a test for viability, have been particularly investigated. It is apparent from published reports that certain enzymes are capable of reducing this salt to the red insoluble formazan and it is assumed that such enzymes are responsible for the reduction of TTC within cells and tissues. In such reactions the temperature is of significance in influencing the speed of the reaction and in completely inactivating the enzymes at high temperatures. There is also some evidence that the colorless solution of TTC can be reduced by light, especially blue light, in the presence of phosphate (Atkinson, Melvin & Fox 1950). Conceivably reduction of TTC by light could occur within living cells.

Tissues of higher plants and of animals, as well as cells of fungi and bacteria, have been reportedly stained by formazan formation; yet almost no reports of critical examination of stained cells have been published. Fat droplets (Seligman & Rutenburg 1951); "at the sites of lipidic components of actively metabolizing cells" (Dufrenoy and Pratt 1948); diffuse reddish pigment (Straus, Cheronis & Straus 1948); deep and homogeneous staining of granules (Fred & Knight 1949); in bacteria, granules similar to mitochondria (Mudd, Brodie, Winterscheid, Hartman, Beutner & McLean 1951); "in some cells it was suggestive that this reaction took place in organoid portions of the cells" (Antopol, Glaubach & Goldman 1948); and, "localized in grana-like regions in the chloroplasts of many kinds of plants and in the cytoplasm of a blue-green alga" (Dyar 1953) suggest the character of published descriptions of intracellular staining by this reaction.

It is evident that fats, cytoplasmic organoids, enzymes diffuse in the cytoplasm or in the organoids, chloroplasts, and phosphates can, in living cells, with proper temperature, and in some cases with light bring about the reduction or become stained by the formazan. It would be expected, therefore, that the formazan would appear in various aspects within cells subjected to tetrazolium. In higher plants the staining is most evident in the most active tissues such as cambium and apical meristems.

[1] From Arapahoe Chemicals, Inc., Boulder, Colorado.

The first part of this paper is a study of intracellular structures stained by the reduced TTC. The second part deals with various aspects of the intracellular reduction and staining reaction in an attempt to evaluate the specificity of TTC as an indicator of the presence of certain enzymes in regard to Stafford's (1951) criticism. She states that there is doubt concerning the specificity of this stain in the light of certain qualifications: that the color change must be due to a specific enzymatic reaction and that the formazan must remain at the site of reduction and not merely accumulate in lipids in which it may be soluble.

Materials. Embryos of the grasses *Zea mays* L. and *Andropogon ischaemum* L., root tips of *Lycopersicon esculentum* Mill., *Pisum sativum* L., *Cooperia pedunculata* Herb., *Tradescantia gigantea* Rose, *Allium cepa* L., and *Z. mays,* mycelium of a species of *Penicillium* and yeast cells were immersed in the TTC solution and examined microscopically.

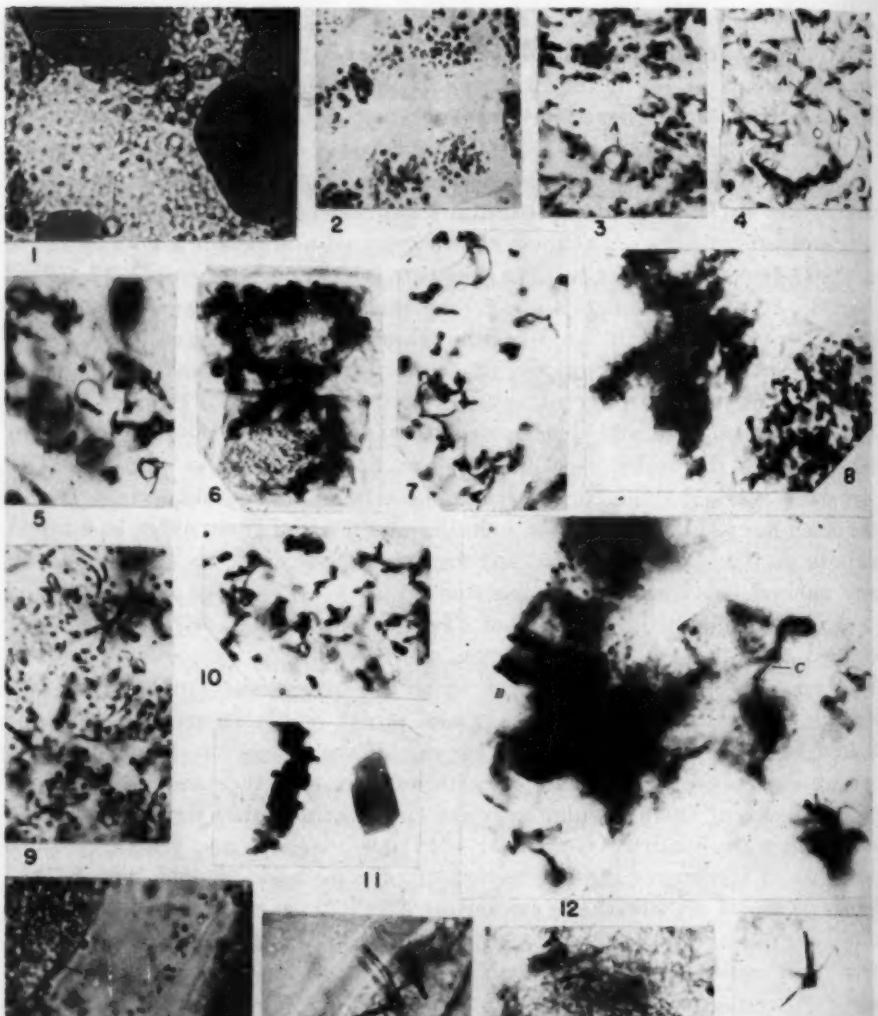
Observations and results. Intracellular staining by TTC. In general, split root tips or embryos exposed ½ to 1 hour in a 0.5–1.0% aqueous solution at room temperature and bright diffuse laboratory light were well stained. The stained root tip material can be cut up and squashed under a cover glass in water, or better, semi-permanent preparations can be made by heating the material (after staining) almost to boiling in a watch glass in a small quantity of 1N HCl for 30–60 seconds and then macerating in a drop of Certo-Karo mixture (10 ml. Certo: 10 ml. Karo: 80 ml. water). After the cover glass is added, the slide is heated almost to boiling and then pressed between absorbent paper. Embryos of grasses need not be split to take up the stain. Small grass seed scarified with fine sand paper will stain in an hour or two and if planted the tops will often grow but the root is usually killed. Root tips, on the other hand, must be cut to stain quickly. However, it was found that onion root tips attached to the bulb can be stained by immersing them in a .01–.5% solution on a laboratory table for a few hours. This treatment kills the roots but concentrations of .001 and .001 will stain some external cells without killing. Staining by this method is not as satisfactory as with split roots in stronger solution. The epidermis and outer layers of the cortex apparently prevent the rapid penetration of tetrazolium. The cells of these layers do not stain well even when the tip is split.

There is a wide range of stained bodies following the TTC treatment. They vary from thread-like bodies to crystals and stained fat droplets. Fat droplets from the embryos of corn (fig. 1) and *Andropogon ischaemum* stain bright red. The stained fat was so abundant that any other stained structures were completely obscured. Stained cells of tomato root tips showed small spherical granules within the cells (fig. 2). This type of structure was one of the commonest in corn, pea, and tomato root tips. The meri-

stematic cells of *Tradescantia* root tips (figs. 3, 4, and 9) contained looped and coiled thread-like structures; small, irregular dark stained objects (most obvious at top of fig. 3); small spheres (fig. 9); sheet-like faintly stained areas; and other structures such as "A" in figures 3 and 4. Cells of root tips *Cooperia pedunculata*, a bulbous species of the Amaryllidaceae, stained by TTC contained in many cells long, looped, thread-like structures also found in *Tradescantia*. Most abundant, however, were the small, irregular-shaped bodies, such as those shown grouped around the nuclei in figure 6. Two adjacent cells of *Cooperia* illustrate the diversity of staining by TTC (fig. 8). One cell is full of the common small, irregular-shaped structures, the other contains only an irregular cloud-like stained area. This latter type of staining was common and often appeared as a sort of dendritic growth. Two rather different crystals are represented in figure 11. Root tip cells of onion showed similar structures and revealed more clearly than other species the rolled sheet-like and brush-like structures (fig. 12). The structure marked "C" (fig. 12) is a sheet 15 to 20 microns long. "B" in the same figure is a side view of a similar sheet. All of these different stained objects may be on one slide of one root tip. In no case was the nucleus or any part of it stained by this reaction.

In some cases, it is evident that structures other than cell organoids are stained (figs. 1, 11 and 14); whereas the thread-like structures (figs. 4, 5, 7, 9, and 10) certainly do appear to be cell organoids. Furthermore, the small, spherical (figs. 2, 9, and 12) and larger irregular granules (figs. 3, 6, and 10) also might represent organoids of the cytoplasm. Figure 9 gives the appearance of a cell full of mitochondria and the thread-like structures, spherical and irregular granules, in this and other figures may well represent mitochondria (Newcomer 1946) and perhaps also plastids.

Stained structures not characteristic of mitochondria such as "A" in figures 3 and 4 are also made evident by TTC. These are ribbon-like bodies, often curved and in figure 4 forked at one end. Broad sheet-like structures that stain rather lightly are commonly seen (fig. 12) as are crystals (fig. 11) and fat droplets (fig. 1). Figure 8 illustrates another type of staining reaction, a diffuse cloud-like mass. These last types (figs. 8, 11, and 12) do not repreesnt typical cell organoids. The rolled sheet-like structures (fig. 12) appear similar in shape and size to those reported by Straus (1950, 1953) in carrot. The structures marked "A" in figures 3 and 4 may correspond to the ribbon-shaped chromoplasts described by Straus. The brush-like structure (fig. 12) consists of a number of curved sheets in end view comparable with an end view of an open book with the leaves separated and curled. The somewhat similar structure (fig. 15) consists of curved filaments attached to one point. Figures 13–16 represent cells that have been treated by a dilute solution (.01%) while the root was intact on the bulb. This



FIGS. 1–16. Photomicrographs of cellular staining by tetrazoliumchloride. Magnification × 1300. FIG. 1. Oil droplets from corn embryo. FIGS. 2–16. Stained structures in root tip cells: of tomato, fig. 2; *Tradescantia gigantea*, figs. 3, 4, and 9; *Cooperia pedunculata*, figs. 5–8, 10 and 11; and onion, figs. 12–16. Forked and curved ribbon-like structures designated "A" in figs. 3 and 4. Thread-like structures, figs. 4, 5, 7, 9 and 10.

treatment resulted in poorer staining and the production of granules on the cell walls (fig. 13) and crystals and brush-like structures (figs. 14–16) within the cells. Crystals are apt to form in cells on slides that have set for a number of hours following these different treatments.

Most of the structures made evident in the cytoplasm by formazan appear to be cell organoids or structures found commonly or occasionally in plant cells. It is expected that mitochondria would be stained since they are reported to contain lipid and probably reducing enzymes and that such substances as Janus Green B, indophenol blue, and osmium tetroxide are reduced by them (Newcomer 1951, Stafford 1951). The rolled sheet-like structures, ribbons, and coiled filaments may compare with structures reported by Straus (1950 and 1953) in carrot. He described the rolled sheet-like structures as being up to 40 by 12 microns in size, "rolled in, curved or undulated in different ways." These structures in carrot are colored and Straus considers them to be chromoplasts. There is some similarity, as an examination of carrot tissue revealed, but it is not close. The brush-like structures are apparently related to the rolled sheets and ribbons. Other structures made evident by the TTC are probably leucoplasts. Dyar (1953) reported chloroplasts or parts of chloroplasts to be stained by a tetrazolium compound.

The stained particles on cell walls appear similar to those reported by Cormack (1948) produced by the reduction of alkaline silver nitrate in roots of white mustard. Crystals, too, are often normal cell constituents but the factors involved in their staining are unknown. The fat droplets found in grass embryos, old mycelia of *Penicillium*, and in yeast cells are probably stained by formazan formed by enzymes in the cytoplasm or at fat-cytoplasm surfaces or by reduction by the fat itself.

It has been noted in higher plants that TTC reduction occurs most obviously and quickly in meristematic cells. This was true in the root tips studied. In the time required to stain these tips older cells of the root were not stained at all. This could be due in part to a higher concentration of reducing enzymes in mitotic cells but certainly older cells must also contain these enzymes. Much longer treatment does produce staining of older cells, but no study was made of these.

Factors involved in reduction and staining. Stafford (1951 has questioned not only the specificity of TTC for reducing enzymes but also whether the formazan in cells is present only at the site of reduction or whether it might be formed in one place and made evident microscopically in some

Small spherical bodies, figs. 2, 3, 9, 12 and 13. Crystals, figs. 11 and 14. Sheet-like structures designated "B" side view and "C" edge view, fig. 12. Brush-like structures, fig. 12 lower right and figs. 15 and 16. Figs 13–16 are of root tip cells stained slowly in .01% solution while attached to bulb and intact. FIG. 13. Granules "G" on cell walls from which the protoplasm "P" has shrunk away.

other place or structure, as for example, dissolved in lipids. For structures such as mitochondria that contain lipids and, presumably, also enzymes, the formazan could be dissolved in the structure that reduced it. If, on the other hand, there are no reducing enzymes on the mitochondria it could be that the reduction took place somewhere else but the formazan dissolved in the lipid of the mitochondria. In that case, the staining of the mitochondria would not be proof that those structures contain reducing enzymes. Dyar (1953) doubts that in the few minutes involved between treatment with TTC and microscopic observation there could be any movement of formazan from one site of reduction to another of accumulation. Another possibility is that enzymes are not always necessary for the reduction of TTC to formazan. Fats, phosphate, crystals, etc., might be able to bring about the reduction independently.

In an effort to determine what might take place the following tests were made. By the use of a Raytheon Co. ultrasonic generator permanent water-oil emulsions were made of raw linseed oil and corn oil. To a portion of each of these emulsions some 1% TTC was added to give a ½% solution. The mixtures were set in a warm place in bright light for one hour at which time both emulsions had become dark pink. There was no staining at the end of the first five minutes but in fifteen minutes the emulsions were definitely pink. Fluorescent laboratory lighting as well as intense incandescent light from a microscope lamp failed to produce any color in two hours. Diffuse daylight from blue sky yielded the results cited above. Direct sunlight will cause a noticeable pink color in two or three minutes. Atkinson, Melvin & Fox (1950) found blue light most active in reduction of TTC.

Microscopic examinations of the stained emulsions revealed that the droplets of linseed oil were only partially stained and then only faint pink. The corn oil emulsion, on the other hand, had all droplets stained bright red as red as the stained oil from squashed stained corn embryos. Corn oil emulsion heated to 90° C. stained in no observable way differently from the unheated emulsion. It is doubtful that in the oils tested there were any enzymes present and it seems reasonable to assume that the oil in blue light can reduce the TTC to formazan.

The second part of the experiment consisted of staining to bright redness a number of *Tradescantia* root tips with TTC. These were then washed in three changes of distilled water for 5 min. A few root tips were then mashed in distilled water. Microscopic examination revealed no oil droplets from the root tip tissue. Another group of the stained and washed root tips were squashed in a drop of the linseed oil-water emulsion for two min. and the mash allowed to set for two additional minutes. Microscopic examination revealed many red oil droplets. It is known that four minutes in labora-

tory light is not enough to cause any TTC still in the root tip tissue to have stained the oil directly. The results indicate the possibility that formazan formed by cellular structures was transferred to the fat droplets of the emulsion. It further indicates that Stafford's doubt of the specificity of the reaction as a test for reducing enzymes is justified.

A further indication that the localized formazan may not always accumulate exactly at the site of reduction is that slides of stained tissue, dead from both TTC and heat, left over night show an increase of certain types of crystals. These could hardly have been formed by enzymes. Another important consideration is the relative insolubility of the red formazan produced by reduction of TTC. A phosphate solution with a small amount of TTC added and allowed to reduce in sunlight for two hours yielded on filtration through filter paper a clear light amber solution. When shaken with a small amount of corn oil and permitted to separate a dark pink or orange oil layer formed. This indicates the possibility that a staining substance produced by the reaction is present in the solution after the formazan formation and might account to some extent for the accumulation of formazan in structures not causing the reduction.

The suggestion of Atkinson, Melvin & Fox (1950) that phosphate in blue light could cause reduction of TTC was investigated. It was found that pure TTC solution in direct sunlight will form formazan but if the pH is raised from 5 to 8 or 9 with KOH the reaction is very quick. Monobasic, dibasic and tribasic phosphate were tested. In direct sunlight the monobasic phosphate causes no more rapid formazan production than pure TTC. Dibasic and especially tribasic phosphate reacted much more rapidly. The indication was that in direct sunlight the pH of the solution is more significant than the phosphate.

Formazan formation by a phosphate solution in sunlight produced a red liquid often with a thin crust on the surface. Microscopic examination revealed very small amorphous masses of formazan in the liquid, about the size of bacteria. Pieces of the surface crust did have an appearance somewhat similar to the sheets (fig. 12) but in no case were they rolled or undulated, only folded or flat with small formazan particles on them and occasionally with holes in the sheets. There were no other similarities between formazan configuration produced by this method and those produced within cells.

The effects of temperature and light on staining. The effect of light on the staining of tissues by tetrazolium has been mentioned by Atkinson, Melvin & Fox (1950) and more recently by Dyar (1953). In general, higher temperatures and higher light intensities give quicker and more intense staining of root tips. Bright incandescent light at 18° C. results in very slow staining but there is almost no staining without light. Bright light

(direct sunlight) and higher temperatures, 37–40° C., affect the reduction strongly so that material stains almost immediately. No light at 37° results in staining about like room temperature and room daylight. It has been found that diffuse daylight from blue sky is much superior to artificial laboratory light. High temperature treatments, above 55° C., that are lethal to soft tissues such as root tips will completely prevent subsequent staining. Root tips heated in water to 80–85° C. for 5 minutes will not stain at all. A 65–70° C. treatment for 10 min. will completely inhibit staining. Root tips heated to 55–60° C. for 10 min. will stain very slightly but 30 min. at that temperature will completely prevent staining. However, root tips treated for 30 min. at 45–50° C. stain as well as untreated root tips. Such heat-killed cells do not stain even when treated with TTC immediately following the lethal treatment.

The mycelium of *Penicillium* appeared submerged in a bottle of 1% TTC in the refrigerator. It grew well at 18° but contained no formazan. A temperature of 37° and bright light was required to stain fat droplets (also stained by Sudan IV) in old hyphae. In yeast cells TTC stains fat droplets better than does Sudan IV.

No apparent differences of stained bodies were found between different treatments of light and temperature, but variation in staining from cell to cell, from tissue to tissue, from root tip to root tip with TTC was wide, ranging from no evident stain through faint pink to dark red and even to dark purple in some cells. The nature of the stained bodies within cells varied widely also, with no apparent correlation with temperature or light.

It has been shown, however (Antopol, Glauback & Goldman 1948, Jensen, Sacks & Baldauski 1951, Roberts 1951) that a considerable number of reducing enzymes of plants are able to reduce TTC. In root tips where there are no oil droplets it is possible that lipids as found in mitochondria and plastids may reduce the TTC. However, there is strong evidence that these structures stained by TTC owe their staining largely to enzymatic reduction of TTC. Root tips heated to 60° C. or more stain not at all even immediately after such killing. The marked sensitivity of the reduction reaction to both temperature and light may well be due to an effect of the active-SH groups of these reducing enzymes (Roberts 1952). Furthermore, the very rapid production of large amounts of formazan in root tip cells at the pH of plant cells can hardly be explained by fat or lipid reduction of TTC which is, evidently, rather slow. It is probable that in many of the cellular structures stained by formazan the reduction is achieved by enzymes in or on the structures and that the formazan accumulates in the lipids of the same structure. However, it is doubtful that TTC reduction should be considered a specific test for reducing enzymes.

SUMMARY

1. Tetrazolium chloride can be reduced to a red insoluble formazan within cells of many plants. This makes visible numerous structures such as mitochondria, plastids, fat droplets, and crystals. The plastids are of various shapes; spheres, coiled filaments, ribbons, and rolled sheets.

2. It was demonstrated that in an emulsion of fat in water produced by ultrasonic vibrations the fat droplets could be stained by formazan.

3. It was further demonstrated that fat droplets of the emulsion could take up formazan previously formed in meristematic cells of root tips.

4. It seems probable, therefore, that tetrazolium chloride is not a specific test for reducing enzymes nor is the occurrence of formazan in a structure proof that it was formed by that structure.

5. Since most plant embryos contain oil which may persist after the death of the embryo (as regards enzymes) and since this oil can be stained under certain conditions, it is not surprising that the TTC test for seed viability is not exact.

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FUNNEL-LEAF OF SPINACH INDUCED BY 2,4-D

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An unusual type of leaf malformation was observed in a commercial planting of winter spinach near Hemet, California (Middleton & Kendrick 1951). The acreage had been planted on different dates. At the time the abnormal plants were seen, the first planting was approximately twelve weeks old, the second ten weeks, and the third about eight weeks. Fifty-one percent of the plants were affected in the first planting, one percent in the second, and none in the third.

FIG. 1, A. Viroflay spinach plant from field collection displaying funnel-leaf of the terminal leaves encasing the apical bud. B. Viroflay spinach showing funnel-leaf malformation induced by exposure to vapors of 2,4-D normal plant on the left.

The disorder is characterized by fusion of the margins of the petioles and part of the lamina of one or more leaves, forming a funnel-like tube which encases the apical bud. Affected leaves are of normal color, show no abnormal venation, and have no tendency toward frenching (fig. 1A). The oldest leaves on affected plants appear normal. The encased apical bud likewise appears normal and frequently grows through the funnel-leaf and produces normal leaves.

Fused petioles were produced in sugar beets by Price, Stewart and Erickson (1950) when sprayed with 2,4-dichlorophenoxyacetic acid (2,4-D) in September or December to prevent bolting. They observed that the seed stalk sometimes emerged from the fused leaf-cylinder. Zimmerman (1951) noted that one of the formative effects of hormone-like growth regulators was a fusion of leaves. Zimmerman and Hitchcock (1941) observed that one of the effects of treating tomatoes by the application of β-naphthoxyacetic acid to the soil was a coalescing of the tips of leaflets which forced the midrib to grow through the opening. They (1942) found that tobacco sprayed with p-chlorophenoxyacetic acid was modified in leaf size, shape, venation and color. Leaves at the growing point were fused and formed a tube which encased the terminal bud. Subsequent leaf growth remained fasciated. They also noted similar malformations on coleus, gynura, and sunflower treated with β-naphthoxyacetic acid.

Materials and methods. Viroflay spinach seedlings were exposed to vapors of the ester of 2,4-dichlorophenoxyacetic acid in petroleum oil carrier to determine whether the emanations from 2,4-D were responsible for funnel-leaf. Two paper towels, 700 sq. cm. each, were saturated with the 2,4-D preparation and hung in a glass-enclosed chamber about 1 cu. m. in size. Seedlings were also placed in the fumigation chamber. Some plants were fumigated continuously for 15, 39, 63 and 87 hours. Other plants were fumigated for fifteen hours then restored to normal atmosphere for nine hours prior to a re-fumigation for fifteen hours. This schedule was repeated so that plants received three and four alternating fifteen-hour exposures of vapors of 2,4-D and nine hours of normal air. Another group of plants was incompletely wetted with a fine mist of 100 p.p.m. 2,4-D and placed out of doors.

Results. Spinach exposed to 15 and 39 hours continuous fumigation developed one set of funnel-shaped leaves thirty days later which were similar to those observed in the field (fig. 1B). Plants exposed for 63 and 87 hours continuous fumigation were killed. Plants exposed to two and three fifteen-hour fumigations also developed funnel-leaf 30 days later. Plants exposed to four fifteen-hour periods of fumigation were killed. Comparable plants exposed only to normal atmosphere produced no abnormal leaves. Plants which had been sprayed with 2,4-D also developed funnel-shaped leaves about thirty days after treatment. The un-sprayed control plants remained healthy. Subsequent growth appeared normal in all affected plants.

Discussion. The spinach leaf malformations induced by sprays and vapors of 2,4-D in the laboratory were similar in some respects to those abnormalities in tobacco, tomato and other plants attributed to p-chlorophenoxyacetic and β-naphthoxyacetic acids by Zimmerman and Hitchcock (1941, 1942). Spinach funnel-leaf also resembles one of the types of growth irregularities in sugar beets sprayed with 2,4-D reported by Price, Stewart

and Erickson (1950). A striking difference in the effect of the 2,4-D on spinach compared to other plants is the absence of most of the usual 2,4-D induced leaf abnormalities consisting of modified venation, vein clearing, distorted growth, and frenching. In spinach the fasciation of one or more leaf petioles and lamina to form a funnel-shaped modified leaf encasing the apical bud appeared to be the only observable malformation. The apical bud developed normally with no fasciation under the conditions of these experiments.

The laboratory-induced funnel-leaf was identical in every respect to the abnormality observed in the commercial spinach. Unsuccessful attempts were made to recover and transmit a virus responsible for funnel-leaf from the commercially affected spinach. It was quite apparent that the funnel-leaf observed in the commercial spinach planting was induced by 2,4-D or some similar growth regulating compound. Since there was no record of an application of such compounds immediately adjacent to the spinach, it is of interest to account for the percentage distribution of affected plants within the three different plantings noted earlier. It was determined that a growth regulator, of some type used for weed control, was applied to a grain field about one mile west of the spinach field. The application was made before the first planting of spinach had emerged from the ground. Formagenic emanations from this compound apparently dissipated slowly for a period of several weeks, affecting 51 percent of the plants in the first planting, one percent in the second, and none in the last.

SUMMARY

1. An uncommon growth abnormality of spinach was induced experimentally by sprays and vapors of 2,4-dichlorophenoxyacetic acid.

2. The term "funnel-leaf" has been applied to the abnormality because of the fusion of the margins of the petiole and a portion of the lamina of one or more leaves forming a funnel-shaped growth encasing the apical bud. No other growth irregularity is produced.

3. Funnel-leaf has been observed in a commercial spinach field and attributed to the emanations of 2,4-D or a similar compound.

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CHROMOSOME NUMBERS AND MORPHOLOGY IN HABRANTHUS AND ZEPHYRANTHES

GERALD E. COE

There are discrepancies in the descriptions of the chromosome number and morphology in several species of *Zephyranthes* and in *Habranthus Andersoni* Herb. (Sato 1942, Flory 1937, 1939, 1940, 1941 and 1948; Nagao & Takusajawa 1932, and Inariyama 1937). The present work was intended to check certain of these discrepancies and to make a more detailed study of the chromosome sets.

Materials and methods. Bulbs of *Zephyranthes Drummondii* D. Don, *Z. brazosensis* Traub, and *Habranthus Andersoni* var. *texanus* Herb. were collected in the vicinity of Austin, Texas. *Z. grandiflora* Lindl. is a cultivated species handled commercially. Specimens of *Z. Traubii* (Hayward) Traub, *Habranthus Andersoni* Herb., and *H. Andersoni* var. *rosea* (Holmberg.) Tindil. were furnished by Dr. H. P. Traub of the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Maryland. Plants of *Zephyranthes longifolia* Hemsley were from a collection made about 60 miles south of Alpine in the Big Bend area of Texas by Dr. W. V. Brown of the University of Texas.

Root tips were excised from actively growing roots and placed in a hydroxyquinoline solution following, with some modifications, the method of Tjio & Levan (1950). The tips were placed in a .002 mol./L. solution of 8-hydroxyquinoline solution for 4 hours at 20° C. After the hydroxyquinoline treatment, the root tips were transferred to a 2 per cent solution of orcein in 45 per cent acetic acid for 24 hours. A root tip about 2 mm. long was then placed in a watchglass with 9 drops of 2 per cent orcein in 45 per cent acetic acid. One drop of 1N HCl was then added. The watchglass was warmed for about 5 seconds over an alcohol lamp and allowed to cool for 5–10 seconds. This heating and cooling was repeated three or four times. The tip was then transferred to a drop of 1 per cent orcein in 45 per cent acetic acid on a glass slide, and the smear was made.

Observations. In most cases the chromosome number in a particular species was found to be constant. Irregularities were found in *Z. longifolia, Z. brazosensis,* and in *Z. Drummondii.* The chromosome numbers observed are presented in table 1.

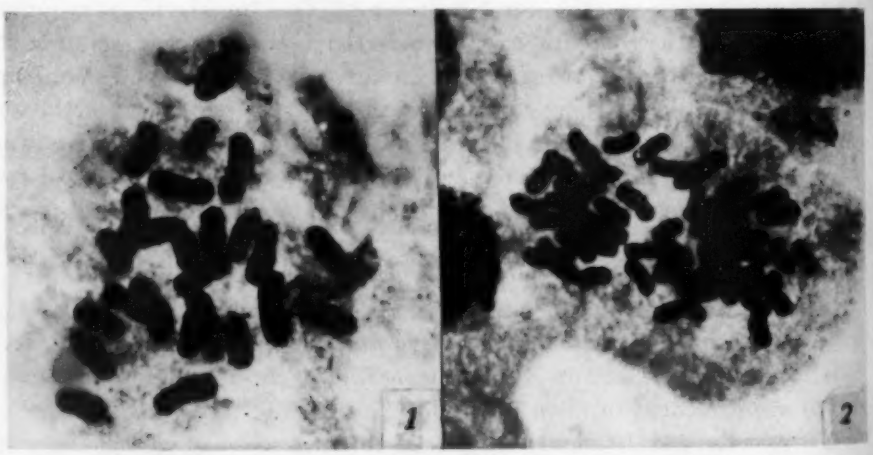
Those cells of *Z. Drummondii* with 24 chromosomes had chromosomes of the same size as those in cells with a somatic number of 48. In *Z. longifolia,* on the other hand, cells with 24 chromosomes had chromosomes with from

TABLE 1. *Chromosome numbers found in some plants of Zephyranthes and Habranthus.*

Species	No. plants examined	Diploid chromosome nos. found in each root tip
Z. Drummondii	9	48
" "	1	48 & 24
Z. brazosensis	9	48
" "	2	58 & 59
" "	1	55 & 56
Z. grandiflora	5	48
Z. Traubii	1	24
Habranthus Andersoni	1	24
H. A. var. *texanus*	5	24
H. A. var. *rosea*	1	24
Z. longifolia	1	46 & 48
" "	1	48, 49, & 50
" "	1	44, 45, 46, & 48
" "	1	44, 45, & 46
" "	1	24, 48, & 49

20 to 30 per cent greater diameters than chromosomes from cells with a somatic number of 44 to 50 chromosomes (figs. 1 and 2).

The idiograms (figs. 3–14) of the species examined revealed the following. First, the variation in length from chromosome to chromosome was so slight that the commonly employed terms long, medium, and short were almost meaningless. Second, the lengths of arms and the positions of the centromeres were such, especially in short chromosomes, that the descriptive terms, median, submedian, subterminal and terminal could not be used. Therefore, the idiograms themselves are presented without the use of such arbitrary terms (figs. 3–14). The relative lengths of the chromosomes in



FIGS. 1–2. Chromosomes in root tip cells of *Zephyranthes longifolia*. FIG. 1. Cell with 24 chromosomes. ×1100. FIG. 2. Cell with 48 chromosomes in the same root tip. ×1100.

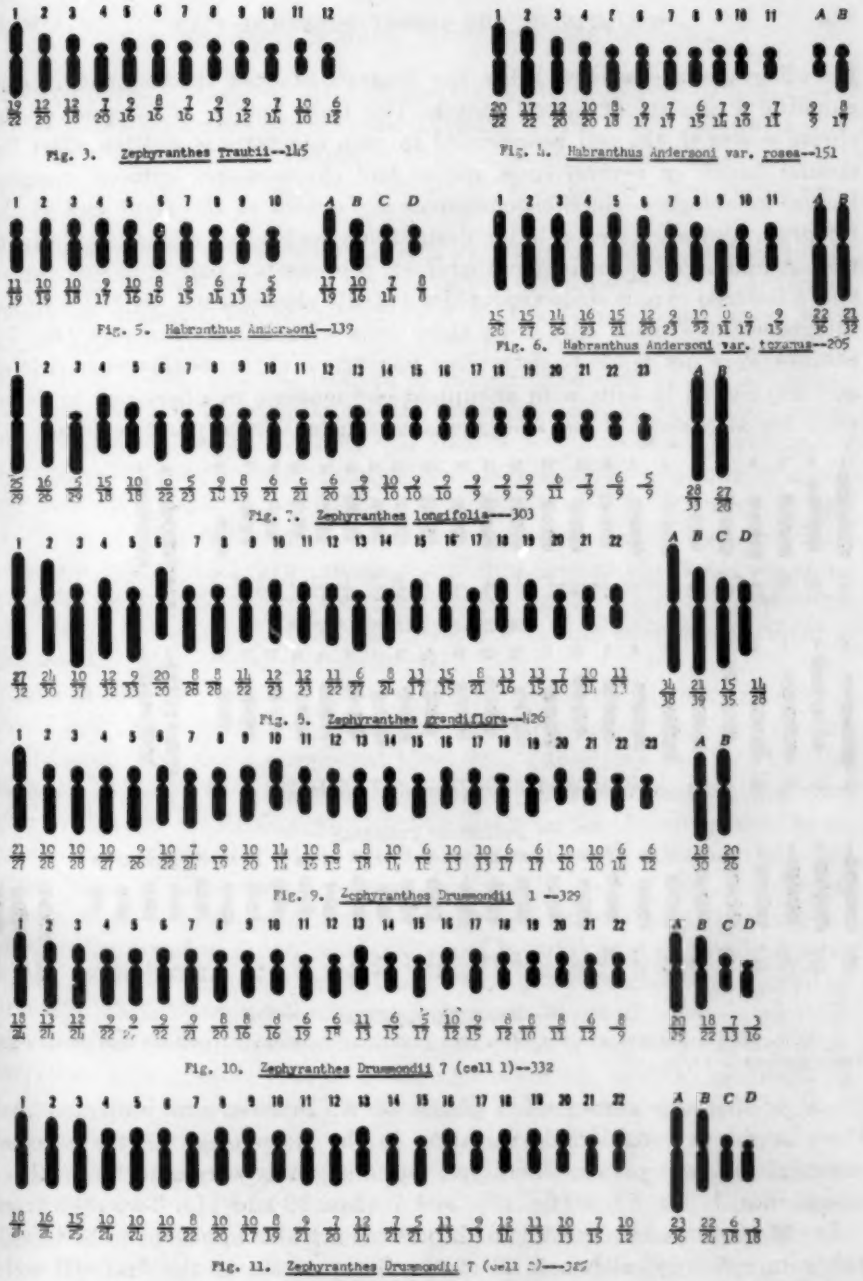


Fig. 3. Zephyranthes Traubii--145

Fig. 4. Habranthus Andersoni var. rosea--151

Fig. 5. Habranthus Andersoni--139

Fig. 6. Habranthus Andersoni var. texanus--205

Fig. 7. Zephyranthes longifolia---303

Fig. 9. Zephyranthes grandiflora---426

Fig. 9. Zephyranthes Drummondii 1 --329

Fig. 10. Zephyranthes Drummondii 7 (cell 1)--332

Fig. 11. Zephyranthes Drummondii 7 (cell 2)---327

FIGS. 3–11. Idiograms of species and plants as indicated. Chromosome numbers above represent a pair that are morphologically similar. Chromosomes lettered above represent single chromosomes that have no morphologically recognizable homologue. Numbers under the chromosomes represent relative lengths of the two arms of the chromosome. The number after the plant name is the length in microns of the sum of the chromosomes of the set.

the idiograms are written below the diagram of each chromosome, .7 mm. equaling 1 micron of actual length. The total length in microns of the chromosomes in the cell represented in each idiogram is written after the species name. In several cases plants had chromosomes without morphological homologues. Such chromosomes are drawn at the right end of the idiogram and are given a letter designation instead of a number designation. A numbered chromosome, therefore, represents a pair of chromosomes, and a lettered chromosome represents a single chromosome. In *Z. longifolia* idiograms were made only from those cells with 48 chromosomes (fig. 7). Studies were not made to determine just which chromosomes were deleted and duplicated in cells with aneuploid chromosome numbers nor to determine for all species if the idiogram was constant from plant to plant.

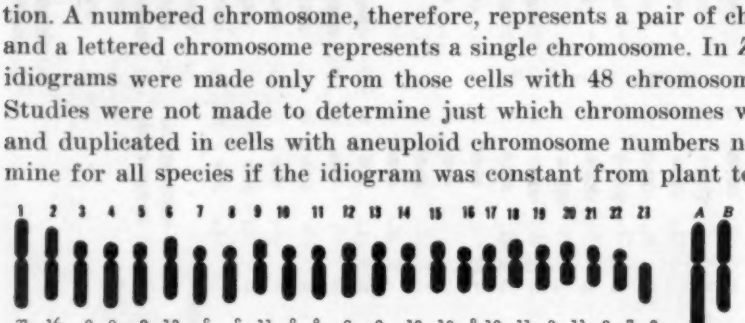


Fig. 12. Zephyranthes Drummondii 5---286

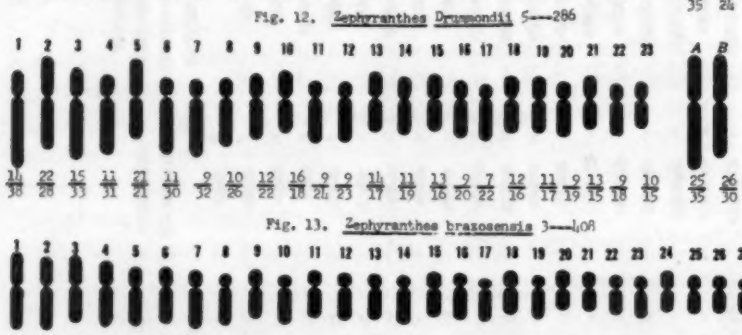


Fig. 13. Zephyranthes brazosensis 3---408

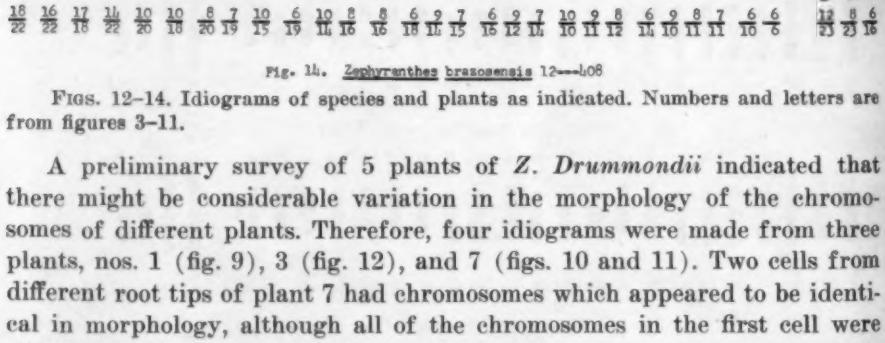


Fig. 14. Zephyranthes brazosensis 12---408

FIGS. 12–14. Idiograms of species and plants as indicated. Numbers and letters are from figures 3–11.

A preliminary survey of 5 plants of *Z. Drummondii* indicated that there might be considerable variation in the morphology of the chromosomes of different plants. Therefore, four idiograms were made from three plants, nos. 1 (fig. 9), 3 (fig. 12), and 7 (figs. 10 and 11). Two cells from different root tips of plant 7 had chromosomes which appeared to be identical in morphology, although all of the chromosomes in the first cell were slightly shorter and narrower than the corresponding chromosomes of the second cell (figs. 10 and 11). On the other hand, when the idiograms prepared from the root tips of plant 7 are compared to those prepared from plant 1 (fig. 9) or plant 5 (fig. 12) or when idiograms prepared from plant

1 are compared to those of plant 5, many differences as well as some similarities in the idiograms are obvious. Throughout the study idiograms prepared from any two or more cells of a single plant appeared to be morphologically identical, whereas idiograms of different plants often varied considerably.

Idiograms of *Z. brazosensis* showed a variation in chromosome morphology among plants with $2n = 48$ similar to the variation found in *Z. Drummondii*. In addition, the chromosomes of *Z. brazosensis* from plants with $2n = 58$ and 59 (fig. 13) were quite different from those of plants with $2n = 48$ (fig. 14). The total lengths of the chromosomes in the idiograms from these two plants, however, was the same. A second check of the chromosome lengths was made, the root tips from a 59-chromosome plant and a 48-chromosome plant being run simultaneously through solutions to eliminate any differences in the pretreatment. Again the total length of the chromosomes in a cell of the 59-chromosome plant was not greater than those in a cell of a 48-chromosome plant, thus confirming the observation that after pretreatment and smearing the average length of a chromosome from the 48-chromosome plant was greater than the average length of a chromosome from a 59-chromosome plant.

Discussion. The plant of *Habranthus Andersoni* on which Sato (1938) worked had chromosomes quite different from the plant which Flory (1938) studied and from the one reported here. Sato's specimen had $2n = 21$; the plants on which Flory worked and those in this study all had $2n = 24$. Sato believed that the *H. Andersoni* with $2n = 21$ was a $2x - 1$ chromosome number and that the species might be closely related to *Cyrtanthus* ($2n = 22$) on the basis of chromosome number and similarities of the karyotypes. Flory gave no details of chromosome morphology other than to say that the chromosomes occur in equal length pairs and to imply that no satellites were present. Sato, on the other hand, found three chromosomes with satellites. Although at least two satellites were faintly visible in *H. Andersoni*, their indistinctness makes it advisable to let their description await better demonstration. It is possible that *H. Andersoni* is similar to *Z. Drummondii* in that there may be considerable variation in chromosome morphology from plant to plant.

The variation in chromosome morphology between the plants of *Habranthus Andersoni* and its two varieties examined in this study is quite evident from their idiograms. Flory (1948) found that the chromosomes of *H. Andersoni* and of *H. Andersoni* var. *texanus* were comparable in length and most arm-length proportions, the main difference between the two chromosome complements being the presence of satellites on the long arm of 3 or 4 chromosomes of *texanus* and the presence of one exceptionally long and one exceptionally short chromosome in *texanus*. Sato (1938) did not recog-

nize these different lengths of chromosomes in *H. Andersoni* var. *texanus*. He states that the chromosomes consist of 16 long and 8 medium length chromosomes. The findings of the present study agree with those of Sato in that the chromosomes of *texanus* are in general of the long and medium length types, whereas *H. Andersoni* and *H. Andersoni* var. *rosea* have only a very few long chromosomes, some medium length ones, and some short ones. The total length of the chromosomes is approximately 50 per cent greater in var. *texanus* than in *H. Andersoni*. The findings in the present study agree with those of Flory in that there is one chromosome definitely longer than any other chromosome in the complement, but a distinctly shorter chromosome was not found. In contrast to *Z. Drummondii* there was no significant variation in the morphology of the chromosomes of the three plants of *H. Andersoni* var. *texanus* studied.

The one specimen of *Z. Traubii* available for study has a karyotype different from that described by Flory (1939). His material had 1 pair of long chromosomes with median constrictions, 1 pair of medium-long chromosomes with submedian constrictions, 8 medium-short pairs with subterminal constrictions, and 2 short pairs with median to submedian constrictions. This description plainly does not fit the idiogram in the present study (fig. 3).

There have been three previous reports on the chromosome numbers in *Z. grandiflra*. Nagao and Takusajawa (1932) found that their specimens had $2n = 46$. Inariyama (1937) and Flory (1941) found that their specimens had $2n = 48$. The material used in the present study had $2n = 48$ (fig. 8). Whether or not the karyotype varies from plant to plant was not determined.

The term aneusomaty was proposed by Duncan (1945) to describe a condition in which occasional cells within a single root tip of *Paphiopedilum wardii* had from 1 to 5 extra chromosomes. In *Z. longifolia* there are not only cells containing extra chromosomes, but also cells deficient for some chromosomes. Perhaps the term aneusomaty could be used in these instances also.

The occurrence of root tip cells with the haploid chromosome number in *Z. Drummondii* might be referable to a somatic reduction similar to those suggested by Huskins and Cheng (1950). Evidences of association of the chromosomes by pairs, however, was not found. The cause of the large size of the chromosomes in cells of *Z. longifolia* with the haploid number (fig. 1) was not ascertained.

The descriptions of the morphology of the chromosomes of *Z. Drummondii* by other investigators do not compare favorably with one another, nor with the idiograms prepared in the present study. It is now evident that the chromosome morphology may vary from plant to plant. Since the

species is apomictic (Coe 1953) the presence of radical differences in chromosome morphology presents few of the genetic difficulties that would occur if sexual reproduction were involved. The behavior of the chromosomes in the microsporocytes suggests the presence of numerous chromosomal aberrations, such as translocations, inversions, and deletions (Coe, 1953).

Apomixis is also present in *Z. brazosensis* and *Habranthus Andersoni* var. *texanus*. (Pace 1913, Coe 1953), both species having plants with aneuploid chromosome numbers. In this group of apomictic species there are marked cytological differences between plants, both as to chromosome morphology as well as numbers. Just how these variations arose is not known but it seems likely that abnormalities of nuclear divisions either in somatic or reproductive tissues are the causes. These cytological aberrations would be sufficient to produce sterility in sexual plants, but apparently in these apomicts a wide range of cytological variation is tolerated.

SUMMARY

1. The karyotype of *Habranthus Andersoni* var. *texanus* was relatively constant in the 3 plants studied.

2. The karyotypes of *Z. Drummondii* and *Z. brazosensis* were constant from cell to cell within a particular plant, but varied considerably from plant to plant. Several chromosome numbers were found in different plants of *Z. brazosensis* and *Z. longifolia*.

3. Aneusomaty was found in *Z. longifolia* as well as an interesting haploid condition in one root tip.

4. The discrepancies which appear in the literature on the karyotype of a particular species of plant may possibly be caused by the methods used in studying the material or in the terminology used in describing the material, but in many cases it may be caused by variation within the species itself.

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SOME NEW OR INTERESTING *PHYSODERMA* SPECIES FROM INDIA

M. J. THIRUMALACHAR AND M. S. PAVGI

Of the several chytridiaceous parasites occurring on phanerogams, the genus *Physoderma* Wallr. has received much attention on account of some of the species being parasitic on plants of economic importance. A synopsis of the genus was published recently by Karling (1950) which has brought together the available information on most of the species. During the course of studies on Indian fungi the authors collected four *Physoderma* species which were either new to science or new records for India. A brief account of the morphological studies carried out is presented here.

***Physoderma graminis* (Büsgen) de Wildeman on *Cynodon dactylon* Pers.** *Cynodon dactylon* Pers., popularly known as Bermuda or Dhup grass, forms an important component of the pastures in India. A *Physoderma* species parasitising the culms and inciting considerable stunting and malformation of the plants was noticed in large patches in the neighbourhood of Banaras, U.P. The infection was always observed in low lying areas which became submerged during the rainy season in July to September. Following the drying up of water in these pools and small puddles there was quick growth of the grass flora. The *Cynodon dactylon* plants in these areas showed severe infection by *Physoderma*, the infected area being restricted to the place which was submerged in water.

The infected plants are conspicuous in the field by their shortened internodes and incurled small leaves which impart a cushionlike or aegigropilous type of habit for the plant. All the shoots developing from the sobole may not show infection, since in some cases a few healthy shoots are also present. However, from the point of infection at a node, all the developing shoots show a systemic type of infection. Infected plants produce normal inflorescences and spikelets in the same way as healthy plants (fig. 1).

Microscopic examination of the diseased plants revealed the characteristic rhizomycelium traversing the cells and the intracellular resting sporangia of the fungus. The rhizomycelia were tenuous, showing intercalary enlargements which were fusiform in shape. The resting sporangia were reddish-brown, thick-walled, smooth, flattened on one side 20–34 × 20–35 μ (fig. 4).

In the aerial portions of the plants, the resting sporangia were mostly distributed within the cortical cells of the stem and a few of the cells of the phloem in the vascular region. There were no resting sporangia within the lamina of the leaves, but there was thick aggregation of the sporangia in the sheathing leaf base and in the portion just above the point of origin of

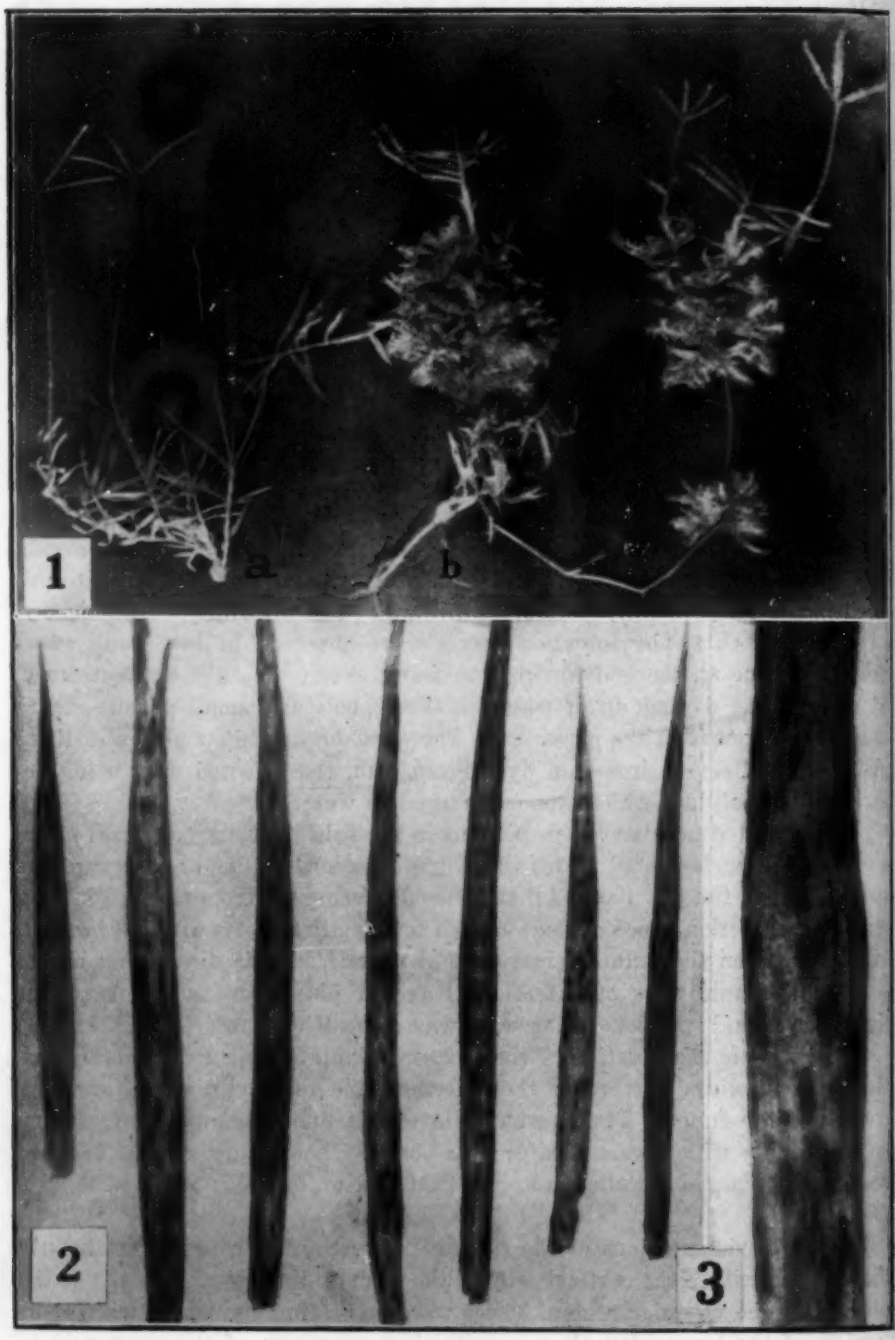
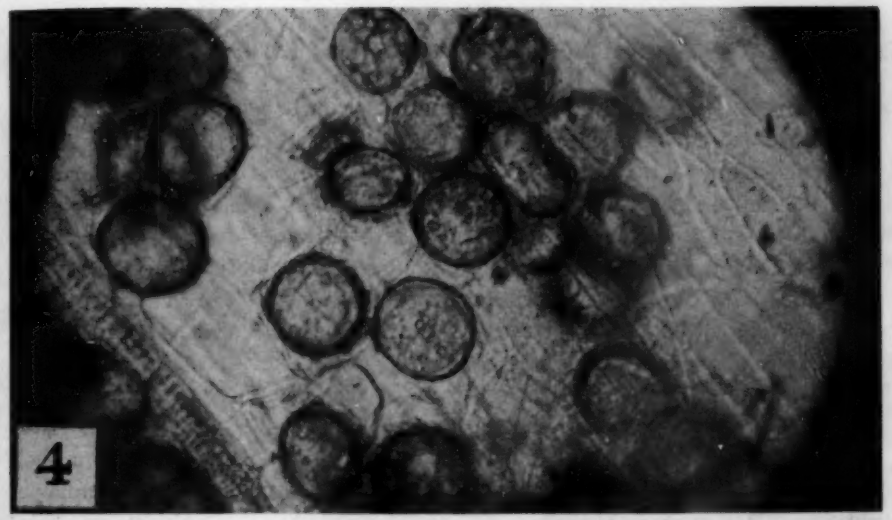
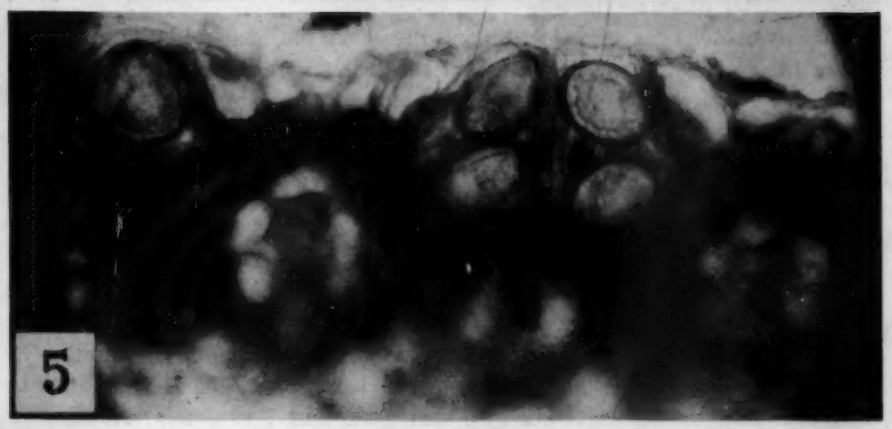


FIG. 1. Healthy (a) and diseased culms (b) of *Cynodon dactylon* infected with *Physoderma graminis*. × 4/5 nat. size. FIG. 2. Diseased leaves of *Dichanthium annulatum*, showing dark elongate spots incited by *Physoderma dichanthicolum*. Approx. nat. size. FIG. 3. Ditto. infection spots enlarged. × 3.





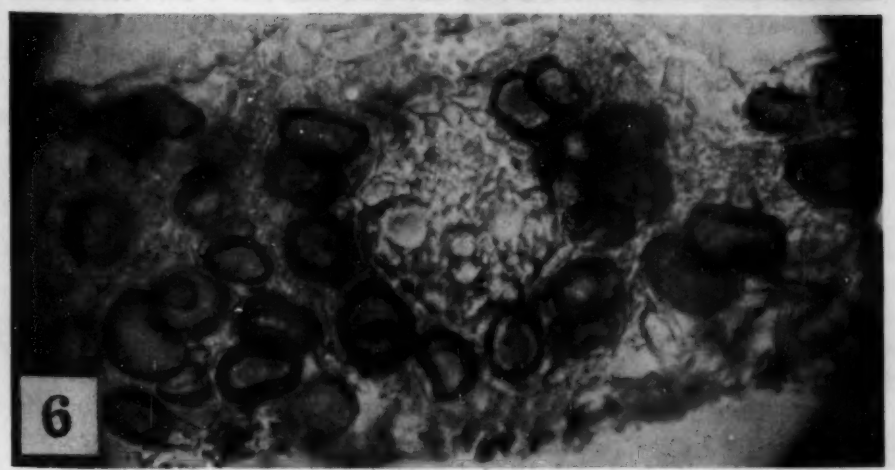


FIG. 4. A typical mass of sporangia of *Physoderma graminis* at the leaf base. About ×300. FIG. 5. T.S. of leaf of *Dichanthium annulatum* showing the sporangia in an enlarged epidermal cell. About ×300. FIG. 6. T.S. of leaf of *Bracharia distachya* showing sporangia in the mesophyll cells around the vascular bundle. About ×250.

the ligule. The presence of small leaves due to improper expansion may be the result of the blocking of transport of food matter to the developing leaves. In sections through the underground soboles the resting sporangia were present in the cortical cells. They were associated with thin-walled structures which appeared to be immature or under-developed stages of the resting sporangia. These may be the same endobiotic thin-walled sporangia described by Gopalakrishnan (1951). Since they may represent only under-developed stages of the resting sporangia in the underground tissues any speculation on their phylogenetic importance seems to be unwarranted.

The germination of the resting sporangia has been observed in very few cases and it conformed to the type observed by Thirumalachar and Dickson (1947) in the same species on *Agropyron repens*. The germination took place by the circumscissile dehiscence and the protrusion of the endosporangium after pushing the lid.

The systemic type of infection produced on the host and the morphology of the resting sporangia indicated that the fungus under study is identical with *Physoderma graminis* which has not been recorded in India so far. In his studies on *Physoderma graminis* on *Agropyron repens*, Gopalakrishnan makes some overbold statements that "the infection of a vascular system by a chytrid is the first report of its kind." It may however, be pointed out that the two species described in the genus *Carpenterella* Tehon are inhabitants of the vascular strands.

An undescribed species of *Physoderma* on *Dichanthium annulatum* (Forsk.) Stapf. On the leaves of *Dichanthium annulatum*, an important forage grass, a species of *Physoderma* was collected during the months of August and September. The infection was localised to small elongated to fusiform spots on the leaves, 2 to 5 mm. long and 1 to 2 mm. broad. The leaves nearer the ground showed more infection than the top leaves. In the initial stages the infected spots appear reddish-green in colour and gradually turn dark brown. The infected leaves showed premature drying up and in the dried leaves the infection spots appear dark in colour (figs. 2 and 3).

Microscopic examination of the infection spots indicated the tenuous rhizomycelium of the fungus and the resting sporangia filling up the host cells. The resting sporangia were produced in any of the epidermal or mesophyll cells, the infected cell usually showing considerable enlargement in size. The resting sporangia were from 2 to 5 in each cell, hemispherical, reddish-brown and flattened on one side. The infected cells enclosing the resting sporangia in due course became filled up with a tannin-like substance which enveloped the sporangia and showed avidity for staining reactions (fig. 5). The individual sporangia could not in consequence be separated easily from the embedding matrix. It seemed therefore probable that these were released only after disintegration of the host tissue. Germination studies of the resting sporangia were not carried out.

As regards the identity, the fungus under study closely resembles *Physoderma paspali* described by Stevenson (1946) on *Paspalum plicatulum* from Puerto Rico. This species has not been included in the monograph of the genus *Physoderma* by Karling (1950). *P. paspali* incites the formation of chocolate-brown to ashen coloured elongated spots on the leaves embedding golden-brown resting sporangia in the leaf tissues. It differs from the species studied by us on *Dichanthium annulatum* in the differences in the size of the resting sporangia and absence of matrix embedding them.

Physoderma dichanthicolum Pavgi and Thirumalachar sp. nov. Incitat formationem macularum in foliis, linearium, 2–5 mm. longarum, 1–2 mm. diam., saepe coalescantium inter se, primo rubrobrunnearum, tandem grisearum vel nigrarum. Rhizomycelium tenue, intracellulare; sporangia quiescentia in cellulis epidermalibus vel mesophyllicis tenuiter hypertrophicis, bina vel terna aggregata, infixa in matricem in cellula hospita, rubro-brunnea, hemisphaerica atque complanata uno latere levia, magnitd. 24–45 × 17.5–31.25 μ, medietate 30 × 22.2 μ. Germinatio haud observata.

Typus lectus in foliis viventibus Dichanthii annulati, in loco Banaras, in provincia U.P., a M. S. Pavgi; nova haec lecta est in loco Patna, in provincia Bihar a M. J. Thirumalachar.

Inciting the formation of leaf spots, linear, 2 to 5 mm. long and 1 to 2 mm. in diameter, often coalescing with each other, reddish-brown at first, later turning grey to black. Rhizomycelium tenuous, intracellular; resting sporangia in slightly hypertrophied epidermal or mesophyli cells, 2 to 5 sporangia clustered, embedded in a matrix within the host cell, reddish-brown hemispherical and flattened on one side, smooth, measuring 24–45 × 17.5–31.25 μ with a mean of 30 × 22.2 μ. Germination not observed.

In living leaves of *Dichanthium annulatum* (Forsk.) Stapf, Banaras, U.P., leg. M. S. Pavgi (Type), Patna, Bihar, leg. M. J. Thirumalachar.

Physoderma species on *Brachiaria distachya* (L.) Haines. During the month of August, a *Physoderma* species was collected on the leaves of *Brachiaria distachya*, a common weed grass in the fields. Only few infected leaves were collected and the collection of more material was made difficult by the presence of *Melanotaenium brachiariae* Viegas which occurred on the same host. The *Physoderma* species incited the paling of the leaves traversed by brown streaks and could be distinguished from the early stages of the infection of the smut *Melanotaenium brachiariae* only with some difficulty.

Sections through the infected leaf revealed the intracellular tenuous rhizomycelium and golden brown resting sporangia. The resting sporangia were mostly distributed within the hyaline cells surrounding the vascular bundles and occasionally within the epidermal cells. Mature resting sporangia are subspherical, flattened on one side, smooth and measure 17–29 × 11–24 μ (Fig. 6). The description of the fungus is as follows:

Physoderma brachiariae Thirumalachar and Pavgi sp. nov. Folia pallescere facit virgis brunneis, tenuiter elevatis; rhizomycelium tenue, intracellulare; sporangia quiescentia intracellularia, 1–3 in singulis cellulis,

fasces vasculares circumdantia, rubro-brunnea, ovata vel subsphaerica, levia, magnit. 17–29 × 11–24 μ. Germinatio haud observata.

Typus lectus in foliis Brachiariae distachyae Haines, in loco Patna, in provincia Bihar, die 4 augusti, 1952 a M. J. Thirumalachar.

Inciting the paling of the leaves with brown slightly raised streaks, rhizomycelium tenuous, intracellular; resting sporangia intracellular, 1 to 3 in each cell surrounding the vascular bundles, reddish-brown, ovate to subspherical, smooth and measuring 17–29 × 11–24 μ. Germination not observed.

In the leaves of *Brachiaria distachya* (L.) Haines, Patna, Bihar, 4th Aug. 1952, leg. M. J. Thirumalachar. (Type).

***Physoderma maculare* Wallr.** On the living leaves of *Alisma reniformis*, Banaras, U.P., 12th Sept. 1952, leg. M. S. Pavgi.

A spot-inciting fungus associated with the smut *Narasimhania alismatis* Pavgi and Thirumalachar was found in one of the collections made near Banaras on *Alisma reniformis*. Examination of the fungus revealed the intracellular resting sporangia which agreed closely with that of *Physoderma maculare* known previously only on *Alisma plantago* and *Echinodorus ranunculoides*. There is no previous record of this fungus in India.

We are grateful to Rev. Father Dr. H. Santapau S. J. Ph.D. F.N.I. for translating diagnoses of the new species into Latin.

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BULLETIN OF THE TORREY BOTANICAL CLUB

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TORREYA

BOOK REVIEW

Fossil plants of the Florissant Beds, Colorado. By Harry D. MacGintie. Carnegie Institution of Washington Publication 599. iii + 198 pp., 75 plates, 1 fig. $5.25 paper; $5.75 cloth. 1953.

Florissant, Colorado is a familiar name to those interested in the study of fossil plants and animals. The past 80 years have seen many papers on the fossils from this area. About 258 fossil plants have been described previously.

The present studies are designed to critically evaluate the species described; to furnish a comprehensive account of the flora; to correlate the fossil species with their modern counterparts; and to use these relationships to postulate climatic and ecological relationships in the Tertiary.

The author describes the geology of this region and locates the fossiliferous sediments in their respective positions. The fossil beds are either lacustrian or fluviatile in origin. They are Oligocene in age and are intermediate between the famous Green River shales (Middle Eocene) and the Pliocene deposits of the Crede and High Plains.

He examined 5300 specimens and calculated the relative abundance of each species. Thirty per cent of the specimens examined are *Fagopsis longifolia* and some nine species make up about 70 per cent of the specimens. The present catalogue lists 114 species of which 20 are described as new. These are distributed as follows: 2 mosses, 2 ferns, 10 gymnosperms, 4 monocotyledonous and 96 dicotyledonous species. This last group is composed of 12 woody vines, 36 shrubs, and 59 trees. The seed plants are grouped into 44 families and 84 genera. The fossils consist of impressions in shale of leaves, seeds, and fruits, although in certain places, petrified stumps of *Sequoia* are common. The fossil leaves were identified by comparison with modern plants and by the aid of competent taxonomists.

The integration of the 258 previously described species into the 114 species recognized in the present publication is indicative of the author's painstaking work in the comparison of the fossils with their modern counterparts and he has the courage to relegate to synonymy the names created by some of the early leaders in this field.

It is interesting to note that about 50 per cent of the living analogues of these fossils grow today in the region from the southern Rockies to San Louis Potosi, in northeastern Mexico and in Texas. About 29 per cent are characteristic of plants at home in western and central China. About the same number are representative of plants now in the Ozarks and the southern Appalachian area.

The ecology of the living representatives of this fossil flora is used to reconstruct the environmental characteristics of the fossil flora. Part of the modern counterparts are members of streamside, lakeside, and low ground habitats; part grow in the transition areas between the low ground and the high ground xeric habitats. The climate of the fossil flora is postulated to be warm temperate with a rainfall not to exceed 20 to 25 inches. The coriaceous texture of the leaves is used to support the concept of a pronounced dry season.

One of the outstanding and praiseworthy features is the nomenclature of the fossils. These are placed in modern genera, with a few exceptions of uncertain affinities, and given specific names. These generic and specific names do not carry the suffixes used to designate the respective organ genera. There are some paleobotanists who prefer to designate the names so that taxonomists will know that the names apply to fossils.

It is to be regretted that cuticle peels to show the structure and distribution of stomates with the epidermal cells are not presented to corroborate the identifications. Likewise, there are no descriptions or figures of pollen, although there are references to their presence in the shales. This specialized study might well be the basis of additional reports on this interesting flora.—CLAIR A. BROWN, Professor of Botany, Louisiana State University, Baton Rouge 3, Louisiana.

Field Trip Reports

Sept. 20. Van Cortlandt Park, N. Y. for a general study of flowers, shrubs, and trees. Attendance 5. Leader, Jane Meyer.

Sept. 26, 27. Point Pleasant to Forked River, N. J. At Wreck Pond, just north of Sea Girt, an extensive colony of the comparatively rare and generally inconspicuous *Limosella subulata* (mudwort) was observed in flower. At Sam's Pond, Point Pleasant, the diminutive *Utricularia gibba* (bladderwort), also of spotty distribution, and the annual *Fuirena pumila*, an occasional salt marsh species, were of special interest. Stops were made at Mantoloking for general strand vegetation; W. Osbornville for the "Schizaea bog;" Lakehurst and Whiting for general pine barren plants; and at Forked River for pine barren, maritime, and coast strip species. At the latter place a nice hybrid oak, x-*Quercus subfalcata* = (*Q. Phellos* × *Q. falcata*) was examined. Attendance 25. Leader, L. E. Hand.

Oct. 11. Greenbrook Sanctuary, New Jersey section Palisades Interstate Park. The sanctuary (about 150 acres) lies between Route 9-W and the famous cliffs. It is located about five miles north of the George Washington Bridge. It is necessary to get advance permission to enter the sanctuary while its development is in progress although ultimately it will be open to all visitors on a regular basis. The forest type on this site is predominantly oak (seven species) and probably represents remnants of the oak-chestnut forest described by E. Lucy Braun. With the chestnut represented only by small sprouts and old stumps, the forest is today largely red oak with admixtures of white oak, sugar maple, black birch, black oak, scarlet oak, hemlock, sweet gum, and sour gum.

The usual fall assortment of *Solidago* and *Aster* were seen flowering and many of the plants in the vegetative stages were identified. It was possible to find three species of of joe-pye weed, *Eupatorium purpurea*, *E. maculata*, and *E. fistulosa*. *Chelone glabra* was still flowering. The moss, *Dicranella heteromalla* was found fruiting near the patrol station beside Green Brook. Heaviest fruiting seed plants were *Apios americana*, the ground nut, seen in the vicinity of the bog and *Strophostyles helola*, another legume, near the entrance to the bog. (See also Bull. Torrey Club **79**: 188. 1952 and **80**: 143. 1953.)

A new reptile record for the sanctuary, the wood turtle was found by the group on this trip.

Checklists are still available listing the plants conspicuous at this time of year together with a resume of the dominant characteristics of this area. They may be obtained from the leader of this trip, Stephen Collins, Department of Botany, Rutgers University, New Brunswick, N. J. Attendance 25.

Oct. 18. Van Cortlandt Park, N. Y. to visit the East Woods section where there is also meadow and swampland. The group was particularly interested in the study of trees. A box-elder, weeping beech, and linden received special attention. Another special was the finding of three varities of Japanese plume grass. Attendance 15. Leader, Jane Meyer.

Oct. 18 Lebanon State Forest, Burlington Co., N. J. Repeated trip of Oct. 26, 1952. (See Bull. Torrey Club **80**: 144.) The second gaging station was in operation and Mr. Henry Barksdale explained some of the problems that had developed and some of the results anticipated. Differences in the vegetation of the burned plots from that a year ago were noted. It was generally agreed that autumn color had rarely been more beautiful in the pine area. Attendance 27. Leaders, Jack McCormick and Murray Buell.

Nov. 15. The Genus Ilex and several other genera that provide shrubs for our gardens were examined on the College of Agriculture grounds at New Brunswick, N. J. A large number of named selections of American, Asiatic, and European species of evergreen holly were seen and compared. Some of the problems of breeding and propagating this dioecious plant were discussed. Attendance 20. Leader, Robert B. Clark.

About Field Trip Reports: With a new season starting, we should like to raise the value of these reports. 1. We wish to put every trip in the record at least for the place, general purpose, and to acknowledge the leader. These records are of historic value. 2. We hope the lunches will be ample and of good quality but they are rarely a valuable record. An exception might be those "living off the country." 3. If the weather

is within the range expected at the season it should be omitted. Bird trips may be exceptions when the weather is a causal factor in numbers of species seen, particularly in unusual circumstances. 4. There should be a reason for each animal or plant named. If it was the biggest daisy ever seen, say so and give its dimensions so readers may know if they ever beat that record. Most conspicuous is usually not enough. The question arises as to what else was there. Sabatia in a bog; was it among cedars, sedges, Sphagnum, rushes, or cat-tails? The absence of a species may be an equally good record. "Among the plants seen" and "the most interesting plants seen" only raise the query, what else was seen or why were these most interesting. Those participating know the answer but the field-going naturalist in Ohio does not know it was the only station in the county. If it was the only station this too should usually be qualified. The only station reported by Britton, or known to remain from those reported by Britton, or known to the leader may bring in further reports. There are many other good reasons. 5. Lists as such are suspect. One doubts their completeness and unless habitat notes are included they turn in queer bedfellows. 6. If there was a purpose and the results warrant it, consider making a short article rather than a field trip report. See Lehr's article in Nov. 1952 number. 7. A survey of an area becomes an important record which will be depended upon for reference. It should be a separate article. All lists should be based upon specimens collected and deposited in an herbarium. If the author has a private herbarium the rare and doubtful species should be verified by another taxonomist and deposited in a public herbarium preferably with duplicates of all the other species. Include adequate descriptions of the area so that changes may be noted with passing years. 8. No more than minor editorial changes will be made in articles without returning them to the author. Galleys will always be sent to authors of articles, book reviews, etc. The editor will make changes as seem warranted in field trip reports without returning them to the author unless asked to so return them. This is in the interest of getting them in print promptly. Galleys of field trips are not sent out to individual authors for the same reason.

Let us have more field trip reports; more prompt field trip reports; and better field trip reports which will be instructive to all our readers instead of just nostalgic to those who participated. We shall also be glad to have short articles or notes based on field trips other than those announced in the Torrey schedule.—J.A.S.

Notes

We have received Anno I—Vol. I—Fasc. I of **"flora salutaris"** (decapitalized), which promises to review some of the medicinal plants in a scientific-popular manner. It must have been some time on the way because it is dated "30-3-1952." The first article is a rather general discussion of utilizing one of our natural resources, the medicinal plants. It covers a good deal of the plant kingdom, more or less bouncing from *Fomes* in the treatment of tuberculosis, to antibiotics, to higher plants; from wild to more or less cultivated "piante medicinali"; from 3000 B. C. to B complex vitamin times. The second article deals specifically with *Rosa canina* L. It is comprehensive, interesting, and draws from the works of several authors. The citations in the text, there is no bibliography, might give one some trouble in locating original papers. A short article discusses the chemurgic movement. Then we come to the cultivation and use of *Salvia Sclarea* L., fourteen pages plus two inserted full-page plates of three half-tones each. *Convallaria majalis* L. is disposed of on two pages, more concentrated but hardly less extensive treatment.

The scientific part, still a review, covers some vitamin research, tissue culture, and a bibliography on Aneurina (Vitamin B_1). Sixteen very short notes reviewing articles, books, etc., bring one to the back cover. Four full-page colored drawings were enclosed but not bound into the journal: *Rosa canina, Salvia Sclarea, Tamus communis,* and *Hypericum perforatum.* Each plate has pertinent botanical, medicinal, and cultural information on reverse side. The colored drawing on front cover conforms to description of *Convallaria majalis* within.

The annual subscription is L. 6000 (call it $10?). Address and make payment to

Flora Salutaris, Instituto ed Orto Botanico dell Universita di Perugia, Casella Postale 204. We found no assurance as to how many numbers you might expect each year.

If you read Italian, or if you wish a botanical stimulus to learn to read Italian, we commend "flora salutaris" to you. If you are learning Italian it will be most helpful to have an Italian speaking coach as we did in the person of Dr. Angelo Lanza di Trabia, of the Romance Language Department, New Jersey College for Women.—J.A.S.

We have learned with regret of the death of Mr. W. Herbert Dole. The Club had regularly been the guest of Mr. and Mrs. Dole for many years to visit their extraordinary fern garden in West Orange. Latest trip report is Bull. Torrey Club 77: 46. 1950. The last visit was in 1950 and apparently was not reported. Mr. Dole's place has been sold and the fate of the garden is uncertain. Perhaps it is as well; no doubt half the fun was in assembling the ferns and working out their establishment.—J.A.S.

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LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITORS OF THE TAXONOMIC INDEX

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